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Docket No.: HYS-26

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE  
PATENT APPLICATION TRANSMITTAL UNDER 37 CFR 1.53



**BOX PATENT APPLICATION**  
Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Transmitted herewith for filing is the patent application of:

Inventor(s): Bryan J. Boyle, George Yeung, Nancy K. Mize, Matthew C. Arterburn, Y. Tom Tang, Chenghua Liu, Radoje T. Drmanac, Menq-Yun Wang, Lichuan Chen, Yea-Huey Yang

Title: METHODS AND MATERIALS RELATING TO LEUCINE-RICH REPEAT PROTEIN-LIKE (LRR PROTEIN-LIKE) POLYPEPTIDES AND POLYNUCLEOTIDES

**1. Type of application**

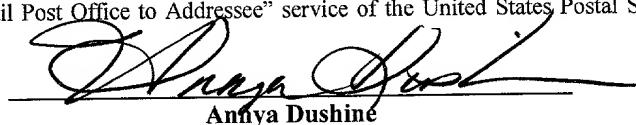
- This is a new application for a
  - Utility patent.  Design patent.
- This is a continuation-in-part application of prior application no. 09/560,875 filed 04/27/00, Attorney Docket No. 787CIP, which is a continuation-in-part application of prior application no. 09/496,914 filed 02/03/00, Attorney Docket No. 787.

**2. Application Papers Enclosed**

- 1 Title Page
- 120 Pages of Specification (excluding Claims, Abstract, Drawings & Sequence Listing)
- 5 Page(s) of Claims
- 1 Page(s) of Abstract
- 5 Sheet(s) of Drawings (Figs. 1A, 1B, 2, 3, 4)
  - Formal  Informal
- 22 Page(s) of Sequence Listing

**CERTIFICATION UNDER 37 CFR 1.10**

I hereby certify that this Patent Application Transmittal and the documents referred to as enclosed therewith are being deposited with the United States Postal Service on September 27, 2000, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231 utilizing the "Express Mail Post Office to Addressee" service of the United States Postal Service under Mailing Label No. EK916749365US

  
Annya Dushine

**3. Oath or Declaration**

- Enclosed
  - Executed by (check all applicable boxes)
  - Inventor(s)
  - Legal representative of inventors(s) (37 CFR 1.42 or 1.43)
  - Joint inventor or person showing a proprietary interest on behalf of inventor who refused to sign or cannot be reached
  - The petition required by 37 CFR 1.47 and the statement required by 37 CFR 1.47 are enclosed. See Item 5D below for fee.
- Unexecuted – the undersigned attorney or agent is authorized to file this application on behalf of the applicant(s). An executed declaration will follow.

**4. Additional Papers Enclosed**

- Preliminary Amendment
- Information Disclosure Statement
- Declaration of Biological Deposit
- Computer readable copy of sequence listing containing nucleotide and/or amino acid sequence and Statement Under 37 CFR § 1.821(f)
- Paper copy of sequence listing identical to computer copy (22 Pages)
- Microfiche computer program
- Verified statement claiming small entity status under 37 CFR 1.9 and 1.27
- Associate Power of Attorney
- Verified translation of a non-English patent application
- Return receipt postcard
- Other \_\_\_\_\_

**5. Priority Applications Under 35 USC 119**

Certified copies of applications from which priority under 35 USC 119 is claimed are listed below and

- are attached.
- will follow.

**6. Filing Fee Calculation (37 CFR 1.16)**

**A.  Utility Application**

CLAIMS AS FILED - INCLUDING PRELIMINARY AMENDMENT (IF ANY)						
			SMALL ENTITY		OTHER THAN A SMALL ENTITY	
	NO. FILED	NO. EXTRA	RATE	FEE	RATE	FEE
BASIC FEE				\$345.00		\$690.00
TOTAL	30-20	= 10	X 9 =	\$90.00	X 18 =	\$0.00
INDEP.	3-3	= 0	X 39 =	\$0.00	X 78 =	\$0.00
<input type="checkbox"/> First Presentation of Multiple Dependent Claim			+ 130 =		+ 260 =	\$0.00
<b>FILING FEE:</b>				<b>\$435.00</b>	<b>OR</b>	<b>\$0.00</b>

**B.  Design Application (\$155.00/\$310.00) Filing Fee: \$ \_\_\_\_\_**

**C.  Plant Application (\$240.00/\$480.00) Filing Fee: \$ \_\_\_\_\_**

**D. Other fees**

- Recording Assignment [Fee -- \$40.00 per assignment] \$ \_\_\_\_\_
- Petition fee for filing by other than all the inventors or person on behalf of the inventor where inventor refused to sign or cannot be reached [Fee -- \$130.00] \$ \_\_\_\_\_
- Other \$ \_\_\_\_\_

**TOTAL FEES ENCLOSED \$ 435.00**

**7. Method of Payments of Fees**

- Enclosed check
- Charge Deposit Account No. 501169. A duplicate copy of this transmittal is enclosed
- Not enclosed

**8. Deposit Account and Refund Authorization**

The Commissioner is hereby authorized to charge payment of any additional fees due or credit any overpayment to Deposit Account No. 501169. A duplicate copy of this transmittal is enclosed.

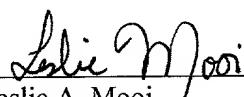
Please refund any overpayment to Hyseq, Inc. at the address below.

Please direct all future correspondence to Leslie A. Mooi at the address below.

Respectfully submitted,

Date: September 27, 2000

By:

  
Leslie A. Mooi

Attorney for Applicants

Registration No.: 37,047

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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant(s) or Patentee(s): Bryan J. Boyle, George Yeung, Nancy K. Mize, Matthew C. Arterburn, Y. Tom Tang, Chenghua Liu, Radoje T. Drmanac, Menq-Yun Wang, Lichuan Chen, Yea-Huey Yang

Application No. or Patent No.: Not Yet Assigned

Filed or Issued: Herewith

For: METHODS AND MATERIALS RELATING TO LEUCINE-RICH REPEAT PROTEIN-LIKE (LRR PROTEIN-LIKE) POLYPEPTIDES AND POLYNUCLEOTIDES

**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS  
(37 CFR § 1.9(f) AND 1.27(c)) - SMALL BUSINESS CONCERN**

I hereby declare that I am

The owner of the small business concern identified below:  
 An official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN: HYSEQ, INC.  
ADDRESS: 670 Almanor Avenue  
Sunnyvale, CA 94085

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 CFR § 121.12, and reproduced in 37 CFR § 1.9(d), for purposes of paying reduced fees under § 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to, and remain with, the small business concern identified above with regard to the invention, entitled METHODS AND MATERIALS RELATING TO LEUCINE-RICH REPEAT PROTEIN-LIKE (LRR PROTEIN-LIKE) POLYPEPTIDES AND POLYNUCLEOTIDES by inventors Bryan J. Boyle, George Yeung, Nancy K. Mize, Matthew C. Arterburn, Y. Tom Tang, Chenghua Liu, Radoje T. Drmanac, Menq-Yun Wang, Lichuan Chen, Yea-Huey Yang, described in

The specification filed herewith.  
 Application Serial No. [ ], filed [Date].  
 Patent No. [ ], issued [Date].

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR § 1.9(c), or by any concern which would not qualify as a small business concern under 37 CFR § 1.9(d) or a nonprofit organization under 37 CFR § 1.9(e).

Full Name: \_\_\_\_\_

Address: \_\_\_\_\_

( ) Individual ( ) Small Business Concern ( ) Nonprofit Organization

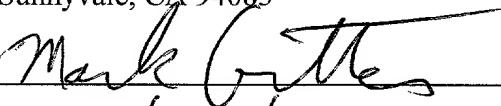
I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate (37 CFR § 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Name of person signing: Mark E. Gitter

Title of person  
other than owner: Chief Financial Officer

Address of person signing: HYSEQ, INC.  
670 Almanor Avenue  
Sunnyvale, CA 94085

Signature: 

Date: 9/27/00

<sup>1</sup>NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR § 1.27)

Our Ref. No.: HYS-26

**METHODS AND MATERIALS RELATING TO LEUCINE-RICH REPEAT  
PROTEIN-LIKE (LRR PROTEIN-LIKE) POLYPEPTIDES AND POLYNUCLEOTIDES**

Express Mail Label No.: EK916749365US

**METHODS AND MATERIALS RELATING TO  
LEUCINE-RICH REPEAT PROTEIN-LIKE (LRR PROTEIN-LIKE)  
POLYPEPTIDES AND POLYNUCLEOTIDES**

5    **1. CROSS REFERENCE TO RELATED APPLICATIONS**

This application is a continuation-in-part application of U.S. Application Serial No. 09/560,875 filed April 27, 2000, entitled "Novel Nucleic Acids and Polypeptides", Attorney Docket No. 787CIP, which in turn is a continuation-in-part application of U.S. Application Serial No. 09/496,914 filed February 03, 2000, entitled "Novel Contigs Obtained from Various Libraries", Attorney Docket No.787, both incorporated herein by reference in their entirety.

15    **2. BACKGROUND**

15    **2.1 TECHNICAL FIELD**

The present invention provides novel polynucleotides and proteins encoded by such polynucleotides, along with uses for these polynucleotides and proteins, for example in therapeutic, diagnostic and research methods. In particular, the invention relates to a novel leucine-rich repeat protein-like (LRR protein-like) polypeptide.

20    **2.2 BACKGROUND ART**

Identified polynucleotide and polypeptide sequences have numerous applications in, for example, diagnostics, forensics, gene mapping; identification of mutations responsible for genetic disorders or other traits, to assess biodiversity, and to produce many other types of data and products dependent on DNA and amino acid sequences. Proteins are known to have biological activity, for example, by virtue of their secreted nature in the case of leader sequence cloning, by virtue of their cell or tissue source in the case of PCR-based techniques, or by virtue of structural similarity to other genes of known biological activity. It is to these polypeptides and the polynucleotides encoding

them that the present invention is directed. In particular, this invention is directed to novel LRR-like polypeptides and polynucleotides.

Leucine-rich repeats are short 20-30 amino acid sequence motifs present in over 100 proteins. LRR proteins are found in all the organisms from bacteria to plants to vertebrates. LRR proteins are involved in protein recognition across a wide range of functions including signal transduction, cell adhesion, development, DNA repair, recombination and transcription, and both plant and animal immune responses. Members of the LRR family of proteins are acid labile subunit (ALS) and glycoprotein (GP) Ib and V.

ALS is involved in the regulation of insulin-like growth factors (IGFs). IGFs regulate diverse cellular processes including cell cycle progression and proliferation, apoptosis, and differentiation. IGFs maintain normal growth and anabolic state of the individual (Heemskerk et al (1999) Cytokine Growth Factor Rev. 10, 5-14). Decreased levels of IGFs in serum are largely responsible for growth retardation and dwarfism. In the immune system, IGFs mediate hematopoiesis, lymphopoiesis, act as survival factors, enhance natural killer cell activity, prime macrophages and neutrophils for free radical production and enhance chemotaxis and differentiation of T cell progenitors. In fetal life, these actions are predominantly carried out by locally produced IGFs. In adult life, IGFs synthesized by the liver are thought to supplement the local IGF production and mediate some of the effects of factors such as growth hormone (GH) and nutrition (Ueki et al (2000) Proc. Natl. Acad. Sci. USA 97, 6868-6873).

Vascular supply, tissue permeability, IGF concentration and most importantly the molecular form of IGF determine the effective IGF availability. Before birth, IGFs exist as binary complexes with members of the family of IGF binding proteins (IGFBPs).

However, after birth, the acid labile subunit (ALS), synthesized by the liver, sequesters most of the IGFs into ternary complexes of about 150 kDa. Unlike the binary complexes that can traverse the capillary endothelia and therefore supply IGFs to the tissues, the ternary complexes are confined to the vasculature. These ternary complexes have extended half-lives and circulate for a longer periods before being broken down by tissue-specific IGFBP proteases to release IGFs. This molecular sequestration allows

IGFs to accumulate in serum to levels reaching 2000-fold higher than that of insulin without causing hypoglycemia.

Acid labile subunit (ALS) is a leucine-rich repeat containing 85 kDa glycoprotein. ALS contains 18 tandem LRR motifs of 24 amino acids each (Janosi et al (1999) J. Biol. Chem. 274, 23328-23332). LRR domains have been shown to participate in protein-protein interaction and may play a role in binding to both IGFs and IGFBPs. Both IGF and ALS gene transcription is regulated by growth hormone (GH) in liver. Increased ALS is detected in cartilaginous and membranous bone, fetal kidney and wound filtrates of adult rats and in human synovial and ovarian fluids.

Because IGFs mediate the effects of growth hormone and other growth factors, effective sequestration of IGFs by ALS is crucial. The dysregulation of ALS may affect heart function, nerve injury, onset of insulin and non-insulin dependent diabetes, muscle disorders, tumor growth, acute and chronic renal failure, stress syndromes, sarcopenia, osteoporosis, inflammation, sepsis and sepsis-induced catabolism, degenerative arthritis, and immune function.

The ability of the body to control the flow of blood following vascular injury is paramount to continued survival. The process of blood clotting and then the subsequent dissolution of the clot, following repair of the injured tissue, is termed hemostasis.

Hemostasis consists of 1) vascular constriction, 2) platelet activation and clumping, 3) formation of thrombus, and 4) clot dissolution following tissue repair. Glycoprotein (GP) Ib-IX-V is a major protein complex on platelets. The initial adhesion of platelets to damaged vessel walls is mediated by binding of von Willebrand factor (vWF) to GP Ib-IX-V complex on platelets. Initial adhesion and binding of other agonists like thrombin, and ADP induce signal transduction events that activate the integrin GP IIbIIIa leading to platelet aggregation and subsequent clot formation.

Both GP Ib and GP V are leucine-rich repeat containing proteins. GP V is cleaved by thrombin from the platelet surface during activation. GP V is shown to be required for the expression of the GP Ib-IX-V complex on the platelet surface (Ramakrishnan et al (1999) Proc. Natl. Acad. Sci. USA 96, 13336-13341). GP Ib on the other hand, has been shown to bind vWF, and thrombin. GP Ib also has been shown to bind Mac-1 on leukocytes and could promote vascular inflammation during thrombosis

(Simon et al (2000) 192, 193-204). The cytoplasmic domains of both GP Ib and GP V bind actin-binding protein and 14-3-3 protein and may serve as a docking site for signaling molecules (Ramakrishnan et al (1999) Proc. Natl. Acad. Sci. USA 96, 13336-13341). The importance of GP Ib-IX-V complex is underscored by the study of Bernard-Soulier syndrome, a bleeding disorder characterized by large platelets that are defective in adhesion to damaged vessel walls (Baumgartner et al (1978) Thromb. Hemostasis 39, 782-783). Recently, mice lacking GP V have been shown to have increased thrombin responsiveness suggesting GP V is a negative modulator of platelet activation (Ramakrishnan et al (1999) Proc. Natl. Acad. Sci. USA 96, 13336-13341).

10 Aberrant expression of GP Ib-IX-V complex can result in severe pathological conditions including hemophilia, bleeding disorders such as Bernard-Soulier syndrome, myocardial infarction, thrombosis, atherosclerosis, angioplasty-related restenosis, glomerular diseases, diabetes, and tumor growth. Manipulating and maintaining the normal expression of these proteins can help develop potential therapies.

15

### **3. SUMMARY OF THE INVENTION**

This invention is based on the discovery of novel LRR protein-like polypeptides, novel isolated polynucleotides encoding such polypeptides, including recombinant DNA molecules, cloned genes or degenerate variants thereof, especially naturally occurring variants such as allelic variants, antisense polynucleotide molecules, and antibodies that specifically recognize one or more epitopes present on such polypeptides, as well as hybridomas producing such antibodies. Specifically, the polynucleotides of the present invention are based on an LRR protein-like polynucleotide isolated from a cDNA library prepared from thymus (Hyseq clone identification numbers 16395938 (SEQ ID NO: 1).

The compositions of the present invention additionally include vectors such as expression vectors containing the polynucleotides of the invention, cells genetically engineered to contain such polynucleotides and cells genetically engineered to express such polynucleotides.

The compositions of the invention provide isolated polynucleotides that include, but are not limited to, a polynucleotide comprising the nucleotide sequence set forth in the SEQ ID NO: 1-3 or 5; or a fragment of SEQ ID NO: 1-3 or 5; a polynucleotide comprising the full length protein coding sequence of the SEQ ID NO: 1-3 or 5 (for example, SEQ ID NO: 4); and a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of any of SEQ ID NO: 1-3 or 5. The polynucleotides of the present invention also include, but are not limited to, a polynucleotide that hybridizes under stringent hybridization conditions to (a) the complement of any of the nucleotide sequences set forth in SEQ ID NO: 1-3 or 5; (b) a nucleotide sequence encoding any of SEQ ID NO: 4 or 6-18; a polynucleotide which is an allelic variant of any polynucleotides recited above having at least 70% polynucleotide sequence identity to the polynucleotides; a polynucleotide which encodes a species homolog (e.g. orthologs) of any of the peptides recited above; or a polynucleotide that encodes a polypeptide comprising a specific domain or truncation of the polypeptide comprising SEQ ID NO: 4.

15 A collection as used in this application can be a collection of only one polynucleotide. The collection of sequence information or unique identifying information of each sequence can be provided on a nucleic acid array. In one embodiment, segments of sequence information are provided on a nucleic acid array to detect the polynucleotide that contains the segment. The array can be designed to detect full-match or mismatch to the polynucleotide that contains the segment. The collection can also be provided in a computer-readable format.

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This invention further provides cloning or expression vectors comprising at least a fragment of the polynucleotides set forth above and host cells or organisms transformed with these expression vectors. Useful vectors include plasmids, cosmids, lambda phage derivatives, phagemids, and the like, that are well known in the art. Accordingly, the invention also provides a vector including a polynucleotide of the invention and a host cell containing the polynucleotide. In general, the vector contains an origin of replication functional in at least one organism, convenient restriction endonuclease sites, and a selectable marker for the host cell. Vectors according to the invention include expression vectors, replication vectors, probe generation vectors, and sequencing vectors. A host cell

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according to the invention can be a prokaryotic or eukaryotic cell and can be a unicellular organism or part of a multicellular organism.

The compositions of the present invention include polypeptides comprising, but not limited to, an isolated polypeptide selected from the group comprising the amino acid sequence of SEQ ID NO: 4 or 6-18; or the corresponding full length or mature protein.

5 Polypeptides of the invention also include polypeptides with biological activity that are encoded by (a) any of the polynucleotides having a nucleotide sequence set forth in the SEQ ID NO: 1-3 or 5; or (b) polynucleotides that hybridize to the complement of the polynucleotides of (a) under stringent hybridization conditions. Biologically or  
10 immunologically active variants of any of the protein sequences listed as SEQ ID NO: 4 or 6-18 and substantial equivalents thereof that retain biological or immunological activity are also contemplated. The polypeptides of the invention may be wholly or partially chemically synthesized but are preferably produced by recombinant means using the genetically engineered cells (e.g. host cells) of the invention.

15 The invention also provides compositions comprising a polypeptide of the invention. Pharmaceutical compositions of the invention may comprise a polypeptide of the invention and an acceptable carrier, such as a hydrophilic, e.g., pharmaceutically acceptable, carrier.

20 The invention also relates to methods for producing a polypeptide of the invention comprising culturing host cells comprising an expression vector containing at least a fragment of a polynucleotide encoding the polypeptide of the invention in a suitable culture medium under conditions permitting expression of the desired polypeptide, and purifying the protein or peptide from the culture or from the host cells. Preferred embodiments include those in which the protein produced by such a  
25 process is a mature form of the protein.

Polynucleotides according to the invention have numerous applications in a variety of techniques known to those skilled in the art of molecular biology. These techniques include use as hybridization probes, use as oligomers, or primers, for PCR, use in an array, use in computer-readable media, use for chromosome and gene mapping, use in the recombinant production of protein, and use in generation of  
30

antisense DNA or RNA, their chemical analogs and the like. For example, when the expression of an mRNA is largely restricted to a particular cell or tissue type, polynucleotides of the invention can be used as hybridization probes to detect the presence of the particular cell or tissue mRNA in a sample using, e.g., *in situ* hybridization.

5

In other exemplary embodiments, the polynucleotides are used in diagnostics as expressed sequence tags for identifying expressed genes or, as well known in the art and exemplified by Vollrath et al., Science 258:52-59 (1992), as expressed sequence tags for physical mapping of the human genome.

10       The polypeptides according to the invention can be used in a variety of conventional procedures and methods that are currently applied to other proteins. For example, a polypeptide of the invention can be used to generate an antibody that specifically binds the polypeptide. Such antibodies, particularly monoclonal antibodies, are useful for detecting or quantitating the polypeptide in tissue. The polypeptides of 15 the invention can also be used as molecular weight markers, and as a food supplement.

Methods are also provided for preventing, treating, or ameliorating a medical condition which comprises the step of administering to a mammalian subject a therapeutically effective amount of a composition comprising a peptide of the present invention and a pharmaceutically acceptable carrier.

20       In particular, the polypeptides and polynucleotides of the invention may be utilized, for example, as potential therapeutics in the treatment of heart failure, nerve injury, insulin and non-insulin dependent diabetes, muscle disorders, tumor growth, acute and chronic renal failure, stress syndromes, sarcopenia, osteoporosis, inflammation, sepsis and sepsis-induced catabolism, degenerative arthritis, blood clotting, and immune function. The polypeptides and polynucleotides of the invention may also be utilized in 25 the treatment of hemophilia, bleeding disorders such as Bernard-Soulier syndrome, myocardial infarction, thrombosis, atherosclerosis, angioplasty-related restenosis, and glomerular diseases. Manipulating and maintaining the normal expression of these proteins can help develop potential therapies.

The methods of the invention also provides methods for the treatment of disorders as recited herein which comprise the administration of a therapeutically effective amount of a composition comprising a polynucleotide or polypeptide of the invention and a pharmaceutically acceptable carrier to a mammalian subject exhibiting symptoms or tendencies related to disorders as recited herein. In addition, the invention encompasses methods for treating diseases or disorders as recited herein comprising the step of administering a composition comprising compounds and other substances that modulate the overall activity of the target gene products and a pharmaceutically acceptable carrier. Compounds and other substances can effect such modulation either on the level of target gene/protein expression or target protein activity. Specifically, methods are provided for preventing, treating or ameliorating a medical condition, including viral diseases, which comprises administering to a mammalian subject, including but not limited to humans, a therapeutically effective amount of a composition comprising a polypeptide of the invention or a therapeutically effective amount of a composition comprising a binding partner of (e.g., antibody specifically reactive for) LRR protein-like polypeptides of the invention. The mechanics of the particular condition or pathology will dictate whether the polypeptides of the invention or binding partners (or inhibitors) of these would be beneficial to the individual in need of treatment.

According to this method, polypeptides of the invention can be administered to produce an *in vitro* or *in vivo* inhibition of cellular function. A polypeptide of the invention can be administered *in vivo* alone or as an adjunct to other therapies. Conversely, protein or other active ingredients of the present invention may be included in formulations of a particular agent to minimize side effects of such an agent.

The invention further provides methods for manufacturing medicaments useful in the above-described methods.

The present invention further relates to methods for detecting the presence of the polynucleotides or polypeptides of the invention in a sample (e.g., tissue or sample). Such methods can, for example, be utilized as part of prognostic and diagnostic

evaluation of disorders as recited herein and for the identification of subjects exhibiting a predisposition to such conditions.

The invention provides a method for detecting a polypeptide of the invention in a sample comprising contacting the sample with a compound that binds to and forms a complex with the polypeptide under conditions and for a period sufficient to form the complex and detecting formation of the complex, so that if a complex is formed, the polypeptide is detected.

The invention also provides kits comprising polynucleotide probes and/or monoclonal antibodies, and optionally quantitative standards, for carrying out methods of the invention. Furthermore, the invention provides methods for evaluating the efficacy of drugs, and monitoring the progress of patients, involved in clinical trials for the treatment of disorders as recited above.

The invention also provides methods for the identification of compounds that modulate (i.e., increase or decrease) the expression or activity of the polynucleotides and/or polypeptides of the invention. Such methods can be utilized, for example, for the identification of compounds that can ameliorate symptoms of disorders as recited herein. Such methods can include, but are not limited to, assays for identifying compounds and other substances that interact with (e.g., bind to) the polypeptides of the invention.

The invention provides a method for identifying a compound that binds to the polypeptide of the present invention comprising contacting the compound with the polypeptide under conditions and for a time sufficient to form a polypeptide/compound complex and detecting the complex, so that if the polypeptide/compound complex is detected, a compound that binds to the polypeptide is identified.

Also provided is a method for identifying a compound that binds to the polypeptide comprising contacting the compound with the polypeptide in a cell for a time sufficient to form a polypeptide/compound complex wherein the complex drives expression of a reporter gene sequence in the cell and detecting the complex by detecting reporter gene sequence expression so that if the polypeptide/compound complex is detected a compound that binds to the polypeptide is identified.

#### **4. BRIEF DESCRIPTION OF THE DRAWINGS**

Figures 1A and 1B show the BLASTX amino acid sequence alignment between the protein encoded by SEQ ID NO: 3 (i.e. SEQ ID NO: 4) LRR protein-like polypeptide and human Garp protein precursor (Garpin) SEQ ID NO: 19, indicating that the two sequences share 51% similarity over 637 amino acid residues of SEQ ID NO: 4 and 38% identity over the same 637 amino acid residues of SEQ ID NO: 4, wherein A=Alanine, C=Cysteine, D=Aspartic Acid, E= Glutamic Acid, F=Phenylalanine, G=Glycine, H=Histidine, I=Isoleucine, K=Lysine, L=Leucine, M=Methionine, N=Asparagine, P=Proline, Q=Glutamine, R=Arginine, S=Serine, T=Threonine, V=Valine, W=Tryptophan, Y=Tyrosine. Gaps are presented as dashes.

Figure 2 shows the BLASTX amino acid sequence alignment between the protein encoded by SEQ ID NO: 3 (i.e. SEQ ID NO: 4) LRR protein-like polypeptide and human insulin-like growth factor binding protein complex acid labile chain precursor protein SEQ ID NO: 20, indicating that the two sequences share 44% similarity over 510 amino acid residues of SEQ ID NO: 4 and 30% identity over the same 510 amino acid residues of SEQ ID NO: 4, wherein A=Alanine, C=Cysteine, D=Aspartic Acid, E= Glutamic Acid, F=Phenylalanine, G=Glycine, H=Histidine, I=Isoleucine, K=Lysine, L=Leucine, M=Methionine, N=Asparagine, P=Proline, Q=Glutamine, R=Arginine, S=Serine, T=Threonine, V=Valine, W=Tryptophan, Y=Tyrosine. Gaps are presented as dashes.

Figure 3 shows the BLASTX amino acid sequence alignment between the protein encoded by SEQ ID NO: 3 (i.e. SEQ ID NO: 4) LRR protein-like polypeptide and human glycoprotein V protein SEQ ID NO: 21, indicating that the two sequences share 45% similarity over 386 amino acid residues of SEQ ID NO: 4 and 32% identity over the same 386 amino acid residues of SEQ ID NO: 4, wherein A=Alanine, C=Cysteine, D=Aspartic Acid, E= Glutamic Acid, F=Phenylalanine, G=Glycine, H=Histidine, I=Isoleucine, K=Lysine, L=Leucine, M=Methionine, N=Asparagine,

P=Proline, Q=Glutamine, R=Arginine, S=Serine, T=Threonine, V=Valine, W=Tryptophan, Y=Tyrosine. Gaps are presented as dashes.

Figure 4 shows the BLASTX amino acid sequence alignment between the protein encoded by SEQ ID NO: 3 (i.e. SEQ ID NO: 4) LRR protein-like polypeptide and synthetic WD-40 domain containing insulin-like growth factor binding protein SEQ ID NO: 22, indicating that the two sequences share 44% similarity over 467 amino acid residues of SEQ ID NO: 4 and 30% identity over the same 467 amino acid residues of SEQ ID NO: 4, wherein A=Alanine, C=Cysteine, D=Aspartic Acid, E= Glutamic Acid, F=Phenylalanine, G=Glycine, H=Histidine, I=Isoleucine, K=Lysine, L=Leucine, M=Methionine, N=Asparagine, P=Proline, Q=Glutamine, R=Arginine, S=Serine, T=Threonine, V=Valine, W=Tryptophan, Y=Tyrosine.

Gaps are presented as dashes.

## 5. DETAILED DESCRIPTION OF THE INVENTION

The LRR protein-like polypeptide of SEQ ID NO: 4 is an approximately 692-amino acid secreted, transmembrane protein with a predicted molecular mass of approximately 77.5 kDa unglycosylated. Protein database searches with the BLASTX algorithm (Altschul S.F. et al., J. Mol. Evol. 36:290-300 (1993) and Altschul S.F. et al., J. Mol. Biol. 21:403-10 (1990), herein incorporated by reference) indicate that SEQ ID NO: 4 is homologous to human ALS protein, human gargin, human platelet glycoprotein V and insulin-like growth factor binding protein.

Figure 1A and Figure 1B show the BLASTX amino acid sequence alignment between the protein encoded by SEQ ID NO: 3 (i.e. SEQ ID NO: 4) LRR protein-like polypeptide and human Garp protein precursor (Gargin) SEQ ID NO: 19, indicating that the two sequences share 51% similarity over 637 amino acid residues of SEQ ID NO: 4 and 38% identity over the same 637 amino acid residues of SEQ ID NO: 4.

Figure 2 shows the BLASTX amino acid sequence alignment between the protein encoded by SEQ ID NO: 3 (i.e. SEQ ID NO: 4) LRR protein-like polypeptide and human insulin-like growth factor binding protein complex acid labile chain precursor protein SEQ ID NO: 20, indicating that the two sequences share 44%

similarity over 510 amino acid residues of SEQ ID NO: 4 and 30% identity over the same 510 amino acid residues of SEQ ID NO: 4.

Figure 3 shows the BLASTX amino acid sequence alignment between the protein encoded by SEQ ID NO: 3 (i.e. SEQ ID NO: 4) LRR protein-like polypeptide 5 and human glycoprotein V protein SEQ ID NO: 21, indicating that the two sequences share 45% similarity over 386 amino acid residues of SEQ ID NO: 4 and 32% identity over the same 386 amino acid residues of SEQ ID NO: 4.

Figure 4 shows the BLASTX amino acid sequence alignment between the protein encoded by SEQ ID NO: 3 (i.e. SEQ ID NO: 4) LRR protein-like polypeptide 10 and synthetic WD-40 domain containing insulin-like growth factor binding protein SEQ ID NO: 22, indicating that the two sequences share 44% similarity over 467 amino acid residues of SEQ ID NO: 4 and 30% identity over the same 467 amino acid residues of SEQ ID NO: 4. The sequences of the present invention are expected to have secreted, transmembrane LRR protein-like activity.

15 A predicted approximately eighteen-residue signal peptide is encoded from approximately residue 1 through residue 18 of SEQ ID NO: 4 (SEQ ID NO: 15). The extracellular portion is useful on its own. This can be confirmed by expression in mammalian cells and sequencing of the cleaved product. The signal peptide region was predicted using the Kyte-Doolittle hydrophobicity prediction algorithm (*J. Mol Biol*, 20 157, pp. 105-31 (1982), incorporated herein by reference). One of skill in the art will recognize that the actual cleavage site may be different than that predicted by the computer program.

A predicted approximately twenty six-residue transmembrane region peptide is encoded from approximately residue 648 through residue 673 of SEQ ID NO: 4 (SEQ 25 ID NO: 16). The transmembrane portion is useful on its own. This can be confirmed by expression in mammalian cells. The transmembrane peptide region was predicted using the Kyte-Doolittle hydrophobicity prediction algorithm (*J. Mol Biol*, 157, pp. 105-31 (1982), incorporated herein by reference).

Using eMATRIX software package (Stanford University, Stanford, CA) (Wu et 30 al., *J. Comp. Biol.*, vol. 6, pp. 219-235 (1999), herein incorporated by reference), LRR

protein-like polypeptide is expected to have nine leucine-rich repeat signatures within residues 378-391, 134-147, 535-548, 375-388, 252-265, 225-238, 560-573, 351-364, and 180-193 of SEQ ID NO: 4 (SEQ ID NO: 6-14). The domains corresponding to SEQ ID NO: 6-14 are as follows wherein A=Alanine, C=Cysteine, D=Aspartic Acid, E=Glutamic Acid, F=Phenylalanine, G=Glycine, H=Histidine, I=Isoleucine, K=Lysine, L=Leucine, M=Methionine, N=Asparagine, P=Proline, Q=Glutamine, R=Arginine, S=Serine, T=Threonine, V=Valine, W=Tryptophan, Y=Tyrosine:

5            Leucine-rich repeat signature  
              LTELDLSHNQLSEL

10          designated as SEQ ID NO: 6 p-value of 5.5e-15, PR00019A (identification number correlating to signature); located at residues 378-391 of SEQ ID NO: 4,  
              Leucine-rich repeat signature  
              LRRLLDLSGNALTED

15          designated as SEQ ID NO: 7 p-value of 3.739e-10, PR00019A (identification number correlating to signature); located at residues 134-147 of SEQ ID NO: 4,  
              Leucine-rich repeat signature  
              FGNLRLDLDLSGNCL

20          designated as SEQ ID NO: 8 p-value of 1.0e-9, PR00019B (identification number correlating to signature); located at residues 535-548 of SEQ ID NO: 4,  
              Leucine-rich repeat signature  
              PGALTELDLSHNQL

25          designated as SEQ ID NO: 9 p-value of 2.44e-9, PR00019B (identification number correlating to signature); located at residues 375-388 of SEQ ID NO: 4,  
              Leucine-rich repeat signature  
              LETLDLSHNQLLFF

30          designated as SEQ ID NO: 10 p-value of 3.333e-9, PR00019A (identification number correlating to signature); located at residues 252-265 of SEQ ID NO: 4,  
              Leucine-rich repeat signature  
              LTRLRVLNVSYNVL

              designated as SEQ ID NO: 11 p-value of 4.96e-9, PR00019B (identification number correlating to signature); located at residues 225-238 of SEQ ID NO: 4,

Leucine-rich repeat signature  
LETLDLRRNNSLTAL  
designated as SEQ ID NO: 12 p-value of 7.0e-9, PR00019A (identification number correlating to signature); located at residues 560-573 of SEQ ID NO: 4,

5 Leucine-rich repeat signature  
MPSLSHNLHQNCL  
designated as SEQ ID NO: 13 p-value of 7.84e-9, PR00019B (identification number correlating to signature); located at residues 351-364 of SEQ ID NO: 4,

10 Leucine-rich repeat signature  
LERLRELDLQRNYI  
designated as SEQ ID NO: 14 p-value of 9.64e-0, PR00019B (identification number correlating to signature); located at residues 180-193 of SEQ ID NO: 4.

In particular, the polypeptides and polynucleotides of the invention may be utilized, for example, as potential therapeutics in the treatment of heart failure, nerve injury, insulin and non-insulin dependent diabetes, muscle disorders, tumor growth, acute and chronic renal failure, stress syndromes, sarcopenia, osteoporosis, inflammation, sepsis and sepsis-induced catabolism and degenerative arthritis, and immune function. The polypeptides and polynucleotides of the invention may also be utilized in the treatment of hemophilia, bleeding disorders such as Bernard-Soulier syndrome, myocardial infarction, thrombosis, atherosclerosis, angioplasty-related restenosis, and glomerular diseases. Manipulating and maintaining the normal expression of these proteins can help develop potential therapies.

## 5.1 DEFINITIONS

25 It must be noted that as used herein and in the appended claims, the singular forms "a", "an" and "the" include plural references unless the context clearly dictates otherwise.

The term "active" refers to those forms of the polypeptide that retain the biologic and/or immunologic activities of any naturally occurring polypeptide.

30 According to the invention, the terms "biologically active" or "biological activity" refer to a protein or peptide having structural, regulatory or biochemical functions of a

naturally occurring molecule. Likewise "biologically active" or "biological activity" refers to the capability of the natural, recombinant or synthetic LRR protein-like peptide, or any peptide thereof, to induce a specific biological response in appropriate animals or cells and to bind with specific antibodies. The term "LRR protein-like biological activity" refers to biological activity that is similar to the biological activity of an LRR protein.

The term "activated cells" as used in this application are those cells which are engaged in extracellular or intracellular membrane trafficking, including the export of secretory or enzymatic molecules as part of a normal or disease process.

The terms "complementary" or "complementarity" refer to the natural binding of polynucleotides by base pairing. For example, the sequence 5'-AGT-3' binds to the complementary sequence 3'-TCA-5'. Complementarity between two single-stranded molecules may be "partial" such that only some of the nucleic acids bind or it may be "complete" such that total complementarity exists between the single stranded molecules. The degree of complementarity between the nucleic acid strands has significant effects on the efficiency and strength of the hybridization between the nucleic acid strands.

The term "embryonic stem cells (ES)" refers to a cell that can give rise to many differentiated cell types in an embryo or an adult, including the germ cells. The term "germ line stem cells (GSCs)" refers to stem cells derived from primordial stem cells that provide a steady and continuous source of germ cells for the production of gametes. The term "primordial germ cells (PGCs)" refers to a small population of cells set aside from other cell lineages particularly from the yolk sac, mesenteries, or gonadal ridges during embryogenesis that have the potential to differentiate into germ cells and other cells. PGCs are the source from which GSCs and ES cells are derived. The PGCs, the GSCs and the ES cells are capable of self-renewal. Thus these cells not only populate the germ line and give rise to a plurality of terminally differentiated cells that comprise the adult specialized organs, but are able to regenerate themselves.

The term "expression modulating fragment," EMF, means a series of nucleotides that modulates the expression of an operably linked ORF or another EMF.

As used herein, a sequence is said to "modulate the expression of an operably linked sequence" when the expression of the sequence is altered by the presence of the EMF. EMFs include, but are not limited to, promoters, and promoter modulating sequences (inducible elements). One class of EMFs is nucleic acid fragments which 5 induce the expression of an operably linked ORF in response to a specific regulatory factor or physiological event.

The terms "nucleotide sequence" or "nucleic acid" or "polynucleotide" or "oligonucleotide" are used interchangeably and refer to a heteropolymer of nucleotides or the sequence of these nucleotides. These phrases also refer to DNA or RNA of 10 genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA) or to any DNA-like or RNA-like material. Where the polynucleotide is RNA, it is understood that the T (thymine) in the sequences provided herein may be substituted with U (uracil). Generally, nucleic acid segments provided by this invention may be assembled 15 from fragments of the genome and short oligonucleotide linkers, or from a series of oligonucleotides, or from individual nucleotides, to provide a synthetic nucleic acid which is capable of being expressed in a recombinant transcriptional unit comprising regulatory elements derived from a microbial or viral operon, or a eukaryotic gene.

The terms "oligonucleotide fragment" or a "polynucleotide fragment", 20 "portion," or "segment" or "probe" or "primer" are used interchangeably and refer to a sequence of nucleotide residues which are at least about 5 nucleotides, more preferably at least about 7 nucleotides, more preferably at least about 9 nucleotides, more preferably at least about 11 nucleotides and most preferably at least about 17 nucleotides. The fragment is preferably less than about 500 nucleotides, preferably less 25 than about 200 nucleotides, more preferably less than about 100 nucleotides, more preferably less than about 50 nucleotides and most preferably less than 30 nucleotides. Preferably the probe is from about 6 nucleotides to about 200 nucleotides, preferably from about 15 to about 50 nucleotides, more preferably from about 17 to 30 nucleotides and most preferably from about 20 to 25 nucleotides. Preferably the fragments can be 30 used in polymerase chain reaction (PCR), various hybridization procedures or

microarray procedures to identify or amplify identical or related parts of mRNA or DNA molecules. A fragment or segment may uniquely identify each polynucleotide sequence of the present invention. Preferably the fragment comprises a sequence substantially similar to a portion of SEQ ID NO: 1-3 or 5.

5 Probes may, for example, be used to determine whether specific mRNA molecules are present in a cell or tissue or to isolate similar nucleic acid sequences from chromosomal DNA as described by Walsh et al. (Walsh, P.S. et al., 1992, PCR Methods Appl 1:241-250). They may be labeled by nick translation, Klenow fill-in reaction, PCR, or other methods well known in the art. Probes of the present  
10 invention, their preparation and/or labeling are elaborated in Sambrook, J. et al., 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, NY; or Ausubel, F.M. et al., 1989, Current Protocols in Molecular Biology, John Wiley & Sons, New York NY, both of which are incorporated herein by reference in their entirety.

15 The nucleic acid sequences of the present invention also include the sequence information from any of the nucleic acid sequences of SEQ ID NO: 1-3 or 5. The sequence information can be a segment of SEQ ID NO: 1-3 or 5 that uniquely identifies or represents the sequence information of SEQ ID NO: 1-3 or 5. One such segment can be a twenty-mer nucleic acid sequence because the probability that a twenty-mer is  
20 fully matched in the human genome is 1 in 300. In the human genome, there are three billion base pairs in one set of chromosomes. Because  $4^{20}$  possible twenty-mers exist, there are 300 times more twenty-mers than there are base pairs in a set of human chromosome. Using the same analysis, the probability for a seventeen-mer to be fully matched in the human genome is approximately 1 in 5. When these segments are used  
25 in arrays for expression studies, fifteen-mer segments can be used. The probability that the fifteen-mer is fully matched in the expressed sequences is also approximately one in five because expressed sequences comprise less than approximately 5% of the entire genome sequence.

Similarly, when using sequence information for detecting a single mismatch, a  
30 segment can be a twenty-five mer. The probability that the twenty-five mer would appear

in a human genome with a single mismatch is calculated by multiplying the probability for a full match ( $1 \div 4^{25}$ ) times the increased probability for mismatch at each nucleotide position ( $3 \times 25$ ). The probability that an eighteen mer with a single mismatch can be detected in an array for expression studies is approximately one in five. The probability 5 that a twenty-mer with a single mismatch can be detected in a human genome is approximately one in five.

The term "open reading frame," ORF, means a series of nucleotide triplets coding for amino acids without any termination codons and is a sequence translatable into protein.

10 The terms "operably linked" or "operably associated" refer to functionally related nucleic acid sequences. For example, a promoter is operably associated or operably linked with a coding sequence if the promoter controls the transcription of the coding sequence. While operably linked nucleic acid sequences can be contiguous and in the same reading frame, certain genetic elements e.g. repressor genes are not 15 contiguously linked to the coding sequence but still control transcription/translation of the coding sequence.

The term "pluripotent" refers to the capability of a cell to differentiate into a number of differentiated cell types that are present in an adult organism. A pluripotent cell is restricted in its differentiation capability in comparison to a totipotent cell.

20 The terms "polypeptide" or "peptide" or "amino acid sequence" refer to an oligopeptide, peptide, polypeptide or protein sequence or fragment thereof and to naturally occurring or synthetic molecules. A polypeptide "fragment," "portion," or "segment" is a stretch of amino acid residues of at least about 5 amino acids, preferably at least about 7 amino acids, more preferably at least about 9 amino acids and most 25 preferably at least about 17 or more amino acids. The peptide preferably is not greater than about 200 amino acids, more preferably less than 150 amino acids and most preferably less than 100 amino acids. Preferably the peptide is from about 5 to about 200 amino acids. To be active, any polypeptide must have sufficient length to display biological and/or immunological activity.

The term "naturally occurring polypeptide" refers to polypeptides produced by cells that have not been genetically engineered and specifically contemplates various polypeptides arising from post-translational modifications of the polypeptide including, but not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation.

5        The term "translated protein coding portion" means a sequence which encodes for the full length protein which may include any leader sequence or a processing sequence.

10      The term "mature protein coding sequence" refers to a sequence which encodes a peptide or protein without any leader/signal sequence. The peptide may have the leader sequences removed during processing in the cell or the protein may have been produced synthetically or using a polynucleotide only encoding for the mature protein coding sequence.

15      The term "derivative" refers to polypeptides chemically modified by such techniques as ubiquitination, labeling (e.g., with radionuclides or various enzymes), covalent polymer attachment such as pegylation (derivatization with polyethylene glycol) and insertion or substitution by chemical synthesis of amino acids such as ornithine, which do not normally occur in human proteins.

20      The term "variant" (or "analog") refers to any polypeptide differing from naturally occurring polypeptides by amino acid insertions, deletions, and substitutions, created using, *e.g.*, recombinant DNA techniques. Guidance in determining which amino acid residues may be replaced, added or deleted without abolishing activities of interest, may be found by comparing the sequence of the particular polypeptide with that of homologous peptides and minimizing the number of amino acid sequence changes made in regions of high homology (conserved regions) or by replacing amino acids with consensus sequence.

25      Alternatively, recombinant variants encoding these same or similar polypeptides may be synthesized or selected by making use of the "redundancy" in the genetic code. Various codon substitutions, such as the silent changes which produce various restriction sites, may be introduced to optimize cloning into a plasmid or viral vector or

expression in a particular prokaryotic or eukaryotic system. Mutations in the polynucleotide sequence may be reflected in the polypeptide or domains of other peptides added to the polypeptide to modify the properties of any part of the polypeptide, to change characteristics such as ligand-binding affinities, interchain affinities, or degradation/turnover rate.

Preferably, amino acid "substitutions" are the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, *i.e.*, conservative amino acid replacements. "Conservative" amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, 10 hydrophilicity, and/or the amphipathic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively 15 charged (acidic) amino acids include aspartic acid and glutamic acid. "Insertions" or "deletions" are preferably in the range of about 1 to 20 amino acids, more preferably 1 to 10 amino acids. The variation allowed may be experimentally determined by systematically making insertions, deletions, or substitutions of amino acids in a 20 polypeptide molecule using recombinant DNA techniques and assaying the resulting recombinant variants for activity.

Alternatively, where alteration of function is desired, insertions, deletions or non-conservative alterations can be engineered to produce altered polypeptides. Such alterations can, for example, alter one or more of the biological functions or biochemical characteristics of the polypeptides of the invention. For example, such 25 alterations may change polypeptide characteristics such as ligand-binding affinities, interchain affinities, or degradation/turnover rate. Further, such alterations can be selected so as to generate polypeptides that are better suited for expression, scale up and the like in the host cells chosen for expression. For example, cysteine residues can be deleted or substituted with another amino acid residue in order to eliminate disulfide 30 bridges.

The terms "purified" or "substantially purified" as used herein denotes that the indicated nucleic acid or polypeptide is present in the substantial absence of other biological macromolecules, *e.g.*, polynucleotides, proteins, and the like. In one embodiment, the polynucleotide or polypeptide is purified such that it constitutes at

5 least 95% by weight, more preferably at least 99% by weight, of the indicated biological macromolecules present (but water, buffers, and other small molecules, especially molecules having a molecular weight of less than 1000 daltons, can be present).

The term "isolated" as used herein refers to a nucleic acid or polypeptide  
10 separated from at least one other component (*e.g.*, nucleic acid or polypeptide) present with the nucleic acid or polypeptide in its natural source. In one embodiment, the nucleic acid or polypeptide is found in the presence of (if anything) only a solvent, buffer, ion, or other components normally present in a solution of the same. The terms "isolated" and "purified" do not encompass nucleic acids or polypeptides present in  
15 their natural source.

The term "recombinant," when used herein to refer to a polypeptide or protein, means that a polypeptide or protein is derived from recombinant (*e.g.*, microbial, insect, or mammalian) expression systems. "Microbial" refers to recombinant polypeptides or proteins made in bacterial or fungal (*e.g.*, yeast) expression systems.

20 As a product, "recombinant microbial" defines a polypeptide or protein essentially free of native endogenous substances and unaccompanied by associated native glycosylation. Polypeptides or proteins expressed in most bacterial cultures, *e.g.*, *E. coli*, will be free of glycosylation modifications; polypeptides or proteins expressed in yeast will have a glycosylation pattern in general different from those expressed in mammalian cells.

25 The term "recombinant expression vehicle or vector" refers to a plasmid or phage or virus or vector, for expressing a polypeptide from a DNA (RNA) sequence. An expression vehicle can comprise a transcriptional unit comprising an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers, (2) a structural or coding sequence which is transcribed into  
30 mRNA and translated into protein, and (3) appropriate transcription initiation and

termination sequences. Structural units intended for use in yeast or eukaryotic expression systems preferably include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, where recombinant protein is expressed without a leader or transport sequence, it may include an amino terminal 5 methionine residue. This residue may or may not be subsequently cleaved from the expressed recombinant protein to provide a final product.

The term "recombinant expression system" means host cells which have stably integrated a recombinant transcriptional unit into chromosomal DNA or carry the recombinant transcriptional unit extrachromosomally. Recombinant expression systems 10 as defined herein will express heterologous polypeptides or proteins upon induction of the regulatory elements linked to the DNA segment or synthetic gene to be expressed. This term also means host cells which have stably integrated a recombinant genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers. Recombinant expression systems as defined herein will 15 express polypeptides or proteins endogenous to the cell upon induction of the regulatory elements linked to the endogenous DNA segment or gene to be expressed. The cells can be prokaryotic or eukaryotic.

The term "secreted" includes a protein that is transported across or through a membrane, including transport as a result of signal sequences in its amino acid 20 sequence when it is expressed in a suitable host cell. "Secreted" proteins include without limitation proteins secreted wholly (e.g., soluble proteins) or partially (e.g., receptors) from the cell in which they are expressed. "Secreted" proteins also include without limitation proteins that are transported across the membrane of the endoplasmic reticulum. "Secreted" proteins are also intended to include proteins containing non- 25 typical signal sequences (e.g. Interleukin-1 Beta, see Krasney, P.A. and Young, P.R. (1992) Cytokine 4(2):134 -143) and factors released from damaged cells (e.g. Interleukin-1 Receptor Antagonist, see Arend, W.P. et. al. (1998) Annu. Rev. Immunol. 16:27-55)

Where desired, an expression vector may be designed to contain a "signal or 30 leader sequence" which will direct the polypeptide through the membrane of a cell.

Such a sequence may be naturally present on the polypeptides of the present invention or provided from heterologous protein sources by recombinant DNA techniques.

The term "stringent" is used to refer to conditions that are commonly understood in the art as stringent. Stringent conditions can include highly stringent  
5 conditions (i.e., hybridization to filter-bound DNA in 0.5 M NaHPO<sub>4</sub>, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C, and washing in 0.1X SSC/0.1% SDS at 68°C), and moderately stringent conditions (i.e., washing in 0.2X SSC/0.1% SDS at 42°C). Other exemplary hybridization conditions are described herein in the examples.

In instances of hybridization of deoxyoligonucleotides, additional exemplary  
10 stringent hybridization conditions include washing in 6X SSC/0.05% sodium pyrophosphate at 37°C (for 14-base oligonucleotides), 48°C (for 17-base oligonucleotides), 55°C (for 20-base oligonucleotides), and 60°C (for 23-base oligonucleotides).

As used herein, "substantially equivalent" can refer both to nucleotide and amino acid sequences, for example a mutant sequence, that varies from a reference sequence by one or more substitutions, deletions, or additions, the net effect of which does not result in an adverse functional dissimilarity between the reference and subject sequences. Typically, such a substantially equivalent sequence varies from one of those listed herein by no more than about 35% (i.e., the number of individual residue substitutions, additions, and/or deletions in a substantially equivalent sequence, as compared to the corresponding reference sequence, divided by the total number of residues in the substantially equivalent sequence is about 0.35 or less). Such a sequence is said to have 65% sequence identity to the listed sequence. In one embodiment, a substantially equivalent, e.g., mutant, sequence of the invention varies from a listed sequence by no more than 30% (70% sequence identity); in a variation of this embodiment, by no more than 25% (75% sequence identity); and in a further variation of this embodiment, by no more than 20% (80% sequence identity) and in a further variation of this embodiment, by no more than 10% (90% sequence identity) and in a further variation of this embodiment, by no more than 5% (95% sequence identity). Substantially equivalent, e.g., mutant, amino acid sequences according to the  
15  
20  
25  
30

invention preferably have at least 80% sequence identity with a listed amino acid sequence, more preferably at least 90% sequence identity. Substantially equivalent nucleotide sequence of the invention can have lower percent sequence identities, taking into account, for example, the redundancy or degeneracy of the genetic code.

5 Preferably, nucleotide sequence has at least about 65% identity, more preferably at least about 75% identity, and most preferably at least about 95% identity. For the purposes of the present invention, sequences having substantially equivalent biological activity and substantially equivalent expression characteristics are considered substantially equivalent. For the purposes of determining equivalence, truncation of the  
10 mature sequence (*e.g.*, via a mutation which creates a spurious stop codon) should be disregarded. Sequence identity may be determined, *e.g.*, using the Jotun Hein method (Hein, J. (1990) Methods Enzymol. 183:626-645). Identity between sequences can also be determined by other methods known in the art, *e.g.* by varying hybridization conditions.

15 The term "totipotent" refers to the capability of a cell to differentiate into all of the cell types of an adult organism.

The term "transformation" means introducing DNA into a suitable host cell so that the DNA is replicable, either as an extrachromosomal element, or by chromosomal integration. The term "transfection" refers to the taking up of an expression vector by a  
20 suitable host cell, whether or not any coding sequences are in fact expressed. The term "infection" refers to the introduction of nucleic acids into a suitable host cell by use of a virus or viral vector.

As used herein, an "uptake modulating fragment," UMF, means a series of  
25 nucleotides which mediate the uptake of a linked DNA fragment into a cell. UMFs can be readily identified using known UMFs as a target sequence or target motif with the computer-based systems described below. The presence and activity of a UMF can be confirmed by attaching the suspected UMF to a marker sequence. The resulting nucleic acid molecule is then incubated with an appropriate host under appropriate conditions and the uptake of the marker sequence is determined. As described above, a UMF will  
30 increase the frequency of uptake of a linked marker sequence.

Each of the above terms is meant to encompass all that is described for each, unless the context dictates otherwise.

## **5.2 NUCLEIC ACIDS OF THE INVENTION**

5       The invention is based on the discovery of a novel secreted LRR protein-like polypeptide, the polynucleotides encoding the LRR protein-like polypeptide and the use of these compositions for the diagnosis, treatment or prevention of cancers and other immunological disorders.

The isolated polynucleotides of the invention include, but are not limited to a  
10 polynucleotide comprising any of the nucleotide sequences of SEQ ID NO: 1-3 or 5; a fragment of SEQ ID NO: 1-3 or 5; a polynucleotide comprising the full length protein coding sequence of SEQ ID NO: 1-3 or 5 (for example SEQ ID NO: 4); and a polynucleotide comprising the nucleotide sequence encoding the mature protein coding sequence of the polynucleotides of any one of SEQ ID NO: 1-3 or 5. The  
15 polynucleotides of the present invention also include, but are not limited to, a polynucleotide that hybridizes under stringent conditions to (a) the complement of any of the nucleotides sequences of the SEQ ID NO: 1-3 or 5; (b) a polynucleotide encoding any one of the polypeptides of SEQ ID NO: 4 or 6-18; (c) a polynucleotide which is an allelic variant of any polynucleotides recited above; (d) a polynucleotide  
20 which encodes a species homolog of any of the proteins recited above; or (e) a polynucleotide that encodes a polypeptide comprising a specific domain or truncation of the polypeptides of SEQ ID NO: 4 or 6-18. Domains of interest may depend on the nature of the encoded polypeptide; e.g., domains in receptor-like polypeptides include ligand-binding, extracellular, transmembrane, or cytoplasmic domains, or combinations  
25 thereof; domains in immunoglobulin-like proteins include the variable immunoglobulin-like domains; domains in enzyme-like polypeptides include catalytic and substrate binding domains; and domains in ligand polypeptides include receptor-binding domains.

The polynucleotides of the invention include naturally occurring or wholly or partially synthetic DNA, e.g., cDNA and genomic DNA, and RNA, e.g., mRNA. The

polynucleotides may include all of the coding region of the cDNA or may represent a portion of the coding region of the cDNA.

The present invention also provides genes corresponding to the cDNA sequences disclosed herein. The corresponding genes can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include the preparation of probes or primers from the disclosed sequence information for identification and/or amplification of genes in appropriate genomic libraries or other sources of genomic materials. Further 5' and 3' sequence can be obtained using methods known in the art. For example, full length cDNA or genomic DNA that corresponds to any of the polynucleotides of the SEQ ID NO: 1-3 or 5 can be obtained by screening appropriate cDNA or genomic DNA libraries under suitable hybridization conditions using any of the polynucleotides of the SEQ ID NO: 1-3 or 5 or a portion thereof as a probe. Alternatively, the polynucleotides of the SEQ ID NO: 1-3 or 5 may be used as the basis for suitable primer(s) that allow identification and/or amplification of genes in appropriate genomic DNA or cDNA libraries.

The nucleic acid sequences of the invention can be assembled from ESTs and sequences (including cDNA and genomic sequences) obtained from one or more public databases, such as dbEST, gbpri, and UniGene. The EST sequences can provide identifying sequence information, representative fragment or segment information, or novel segment information for the full-length gene.

The polynucleotides of the invention also provide polynucleotides including nucleotide sequences that are substantially equivalent to the polynucleotides recited above. Polynucleotides according to the invention can have, *e.g.*, at least about 65%, at least about 70%, at least about 75%, at least about 80%, more typically at least about 90%, and even more typically at least about 95%, sequence identity to a polynucleotide recited above.

Included within the scope of the nucleic acid sequences of the invention are nucleic acid sequence fragments that hybridize under stringent conditions to any of the nucleotide sequences of the SEQ ID NO: 1-3 or 5, or complements thereof, which fragment is greater than about 5 nucleotides, preferably 7 nucleotides, more preferably

greater than 9 nucleotides and most preferably greater than 17 nucleotides. Fragments of, e.g. 15, 17, or 20 nucleotides or more that are selective for (i.e. specifically hybridize to any one of the polynucleotides of the invention) are contemplated. Probes capable of specifically hybridizing to a polynucleotide can differentiate polynucleotide sequences of the invention from other polynucleotide sequences in the same family of genes or can differentiate human genes from genes of other species, and are preferably based on unique nucleotide sequences.

The sequences falling within the scope of the present invention are not limited to these specific sequences, but also include allelic and species variations thereof. Allelic and species variations can be routinely determined by comparing the sequence provided in SEQ ID NO: 1-3 or 5, a representative fragment thereof, or a nucleotide sequence at least 90% identical, preferably 95% identical, to SEQ ID NO: 1-3 or 5 with a sequence from another isolate of the same species. Furthermore, to accommodate codon variability, the invention includes nucleic acid molecules coding for the same amino acid sequences as do the specific ORFs disclosed herein. In other words, in the coding region of an ORF, substitution of one codon for another codon that encodes the same amino acid is expressly contemplated.

The nearest neighbor result for the nucleic acids of the present invention, including SEQ ID NO: 1-3 or 5, can be obtained by searching a database using an algorithm or a program. Preferably, a BLAST which stands for Basic Local alignment Search Tool is used to search for local sequence alignments (Altshul, S.F. J Mol. Evol. 36 290-300 (1993) and Altschul S.F. et al. J. Mol. Biol. 21:403-410 (1990))

Species homologs (or orthologs) of the disclosed polynucleotides and proteins are also provided by the present invention. Species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source from the desired species.

The invention also encompasses allelic variants of the disclosed polynucleotides or proteins; that is, naturally-occurring alternative forms of the isolated polynucleotide which also encode proteins which are identical, homologous or related to that encoded by the polynucleotides.

The nucleic acid sequences of the invention are further directed to sequences which encode variants of the described nucleic acids. These amino acid sequence variants may be prepared by methods known in the art by introducing appropriate nucleotide changes into a native or variant polynucleotide. There are two variables in  
5 the construction of amino acid sequence variants: the location of the mutation and the nature of the mutation. Nucleic acids encoding the amino acid sequence variants are preferably constructed by mutating the polynucleotide to encode an amino acid sequence that does not occur in nature. These nucleic acid alterations can be made at sites that differ in the nucleic acids from different species (variable positions) or in  
10 highly conserved regions (constant regions). Sites at such locations will typically be modified in series, *e.g.*, by substituting first with conservative choices (*e.g.*, hydrophobic amino acid to a different hydrophobic amino acid) and then with more distant choices (*e.g.*, hydrophobic amino acid to a charged amino acid), and then deletions or insertions may be made at the target site. Amino acid sequence deletions  
15 generally range from about 1 to 30 residues, preferably about 1 to 10 residues, and are typically contiguous. Amino acid insertions include amino- and/or carboxyl-terminal fusions ranging in length from one to one hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Intrasequence insertions may range generally from about 1 to 10 amino residues, preferably from 1 to  
20 5 residues. Examples of terminal insertions include the heterologous signal sequences necessary for secretion or for intracellular targeting in different host cells and sequences such as FLAG or poly-histidine sequences useful for purifying the expressed protein.

In a preferred method, polynucleotides encoding the novel amino acid sequences  
25 are changed via site-directed mutagenesis. This method uses oligonucleotide sequences to alter a polynucleotide to encode the desired amino acid variant, as well as sufficient adjacent nucleotides on both sides of the changed amino acid to form a stable duplex on either side of the site being changed. In general, the techniques of site-directed mutagenesis are well known to those of skill in the art and this technique is exemplified  
30 by publications such as, Edelman et al., *DNA* 2:183 (1983). A versatile and efficient

method for producing site-specific changes in a polynucleotide sequence was published by Zoller and Smith, *Nucleic Acids Res.* 10:6487-6500 (1982). PCR may also be used to create amino acid sequence variants of the novel nucleic acids. When small amounts of template DNA are used as starting material, primer(s) that differs slightly in  
5 sequence from the corresponding region in the template DNA can generate the desired amino acid variant. PCR amplification results in a population of product DNA fragments that differ from the polynucleotide template encoding the polypeptide at the position specified by the primer. The product DNA fragments replace the corresponding region in the plasmid and this gives a polynucleotide encoding the  
10 desired amino acid variant.

A further technique for generating amino acid variants is the cassette mutagenesis technique described in Wells et al., *Gene* 34:315 (1985); and other mutagenesis techniques well known in the art, such as, for example, the techniques in Sambrook et al., *supra*, and *Current Protocols in Molecular Biology*, Ausubel et al.  
15 Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be used in the practice of the invention for the cloning and expression of these novel nucleic acids. Such DNA sequences include those which are capable of hybridizing to the appropriate novel nucleic acid sequence under stringent conditions.

20 Polynucleotides encoding preferred polypeptide truncations of the invention can be used to generate polynucleotides encoding chimeric or fusion proteins comprising one or more domains of the invention and heterologous protein sequences.

The polynucleotides of the invention additionally include the complement of any of the polynucleotides recited above. The polynucleotide can be DNA (genomic,  
25 cDNA, amplified, or synthetic) or RNA. Methods and algorithms for obtaining such polynucleotides are well known to those of skill in the art and can include, for example, methods for determining hybridization conditions that can routinely isolate polynucleotides of the desired sequence identities.

In accordance with the invention, polynucleotide sequences comprising the  
30 mature protein coding sequences corresponding to any one of SEQ ID NO: 4 or 6-18,

or functional equivalents thereof, may be used to generate recombinant DNA molecules that direct the expression of that nucleic acid, or a functional equivalent thereof, in appropriate host cells. Also included are the cDNA inserts of any of the clones identified herein.

5 A polynucleotide according to the invention can be joined to any of a variety of other nucleotide sequences by well-established recombinant DNA techniques (see Sambrook J et al. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, NY). Useful nucleotide sequences for joining to polynucleotides include an assortment of vectors, e.g., plasmids, cosmids, lambda phage derivatives, 10 phagemids, and the like, that are well known in the art. Accordingly, the invention also provides a vector including a polynucleotide of the invention and a host cell containing the polynucleotide. In general, the vector contains an origin of replication functional in at least one organism, convenient restriction endonuclease sites, and a selectable marker for the host cell. Vectors according to the invention include 15 expression vectors, replication vectors, probe generation vectors, and sequencing vectors. A host cell according to the invention can be a prokaryotic or eukaryotic cell and can be a unicellular organism or part of a multicellular organism.

The present invention further provides recombinant constructs comprising a nucleic acid having any of the nucleotide sequences of the SEQ ID NO: 1-3 or 5 or a fragment thereof or any other polynucleotides of the invention. In one embodiment, the recombinant constructs of the present invention comprise a vector, such as a plasmid or viral vector, into which a nucleic acid having any of the nucleotide sequences of the SEQ ID NO: 1-3 or 5 or a fragment thereof is inserted, in a forward or reverse orientation. In the case of a vector comprising one of the ORFs of the present 25 invention, the vector may further comprise regulatory sequences, including for example, a promoter, operably linked to the ORF. Large numbers of suitable vectors and promoters are known to those of skill in the art and are commercially available for generating the recombinant constructs of the present invention. The following vectors are provided by way of example. Bacterial: pBs, phagescript, PsiX174, pBluescript 30 SK, pBs KS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene); pTrc99A, pKK223-3,

pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWLneo, pSV2cat, pOG44, PXTI, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia).

The isolated polynucleotide of the invention may be operably linked to an expression control sequence such as the pMT2 or pED expression vectors disclosed in 5 Kaufman et al., *Nucleic Acids Res.* 19, 4485-4490 (1991), in order to produce the protein recombinantly. Many suitable expression control sequences are known in the art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman, *Methods in Enzymology* 185, 537-566 (1990). As defined herein "operably linked" means that the isolated polynucleotide of the invention and an 10 expression control sequence are situated within a vector or cell in such a way that the protein is expressed by a host cell which has been transformed (transfected) with the ligated polynucleotide/expression control sequence.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two 15 appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda PR, and trc. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art. Generally, recombinant 20 expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of *E. coli* and *S. cerevisiae* TRP1 gene, and a promoter derived from a highly expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), 25 a-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an amino terminal 30 identification peptide imparting desired characteristics, e.g., stabilization or simplified

purification of expressed recombinant product. Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic  
5 selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium* and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, although others may also be employed as a matter of choice.

10 As a representative but non-limiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM 1  
15 (Promega Biotech, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed. Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced or derepressed by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an  
20 additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Polynucleotides of the invention can also be used to induce immune responses. For example, as described in Fan et al., *Nat. Biotech.* 17:870-872 (1999), incorporated herein by reference, nucleic acid sequences encoding a polypeptide may be used to  
25 generate antibodies against the encoded polypeptide following topical administration of naked plasmid DNA or following injection, and preferably intramuscular injection of the DNA. The nucleic acid sequences are preferably inserted in a recombinant expression vector and may be in the form of naked DNA.

30 **5.3 HOSTS**

The present invention further provides host cells genetically engineered to contain the polynucleotides of the invention. For example, such host cells may contain nucleic acids of the invention introduced into the host cell using known transformation, transfection or infection methods. The present invention still further provides host cells 5 genetically engineered to express the polynucleotides of the invention, wherein such polynucleotides are in operative association with a regulatory sequence heterologous to the host cell which drives expression of the polynucleotides in the cell.

Knowledge of LRR protein-like DNA sequences allows for modification of cells to permit, or increase, expression of LRR protein-like polypeptide. Cells can be 10 modified (e.g., by homologous recombination) to provide increased LRR protein-like polypeptide expression by replacing, in whole or in part, the naturally occurring LRR protein-like promoter with all or part of a heterologous promoter so that the cells LRR protein-like polypeptide is expressed at higher levels. The heterologous promoter is inserted in such a manner that it is operatively linked to LRR protein-like encoding 15 sequences. See, for example, PCT International Publication No. WO94/12650, PCT International Publication No. WO92/20808, and PCT International Publication No. WO91/09955. It is also contemplated that, in addition to heterologous promoter DNA, amplifiable marker DNA (e.g., ada, dhfr, and the multifunctional CAD gene which encodes carbamyl phosphate synthase, aspartate transcarbamylase, and dihydroorotate) 20 and/or intron DNA may be inserted along with the heterologous promoter DNA. If linked to the LRR protein-like coding sequence, amplification of the marker DNA by standard selection methods results in co-amplification of the LRR protein-like coding sequences in the cells.

The host cell can be a higher eukaryotic host cell, such as a mammalian cell, a 25 lower eukaryotic host cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the recombinant construct into the host cell can be effected by calcium phosphate transfection, DEAE, dextran-mediated transfection, or electroporation (Davis, L. et al., *Basic Methods in Molecular Biology* (1986)). The host cells containing one of the polynucleotides of the invention, can be 30 used in conventional manners to produce the gene product encoded by the isolated

fragment (in the case of an ORF) or can be used to produce a heterologous protein under the control of the EMF.

Any host/vector system can be used to express one or more of the ORFs of the present invention. These include, but are not limited to, eukaryotic hosts such as HeLa cells, Cv-1 cell, COS cells, 293 cells, and Sf9 cells, as well as prokaryotic host such as *E. coli* and *B. subtilis*. The most preferred cells are those which do not normally express the particular polypeptide or protein or which expresses the polypeptide or protein at low natural level. Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., in Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, New York (1989), the disclosure of which is hereby incorporated by reference.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell 23:175 (1981). Other cell lines capable of expressing a compatible vector are, for example, the C127, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from *in vitro* culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK or Jurkat cells. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements. Recombinant polypeptides and proteins produced in bacterial culture are usually isolated by initial extraction from cell pellets, followed by one or more salting-

out, aqueous ion exchange or size exclusion chromatography steps. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of proteins can be disrupted 5 by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

Alternatively, it may be possible to produce the protein in lower eukaryotes such as yeast or insects or in prokaryotes such as bacteria. Potentially suitable yeast strains include *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces* strains, 10 *Candida*, or any yeast strain capable of expressing heterologous proteins. Potentially suitable bacterial strains include *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, or any bacterial strain capable of expressing heterologous proteins. If the protein is made in yeast or bacteria, it may be necessary to modify the protein produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in 15 order to obtain the functional protein. Such covalent attachments may be accomplished using known chemical or enzymatic methods.

In another embodiment of the present invention, cells and tissues may be engineered to express an endogenous gene comprising the polynucleotides of the invention under the control of inducible regulatory elements, in which case the 20 regulatory sequences of the endogenous gene may be replaced by homologous recombination. As described herein, gene targeting can be used to replace a gene's existing regulatory region with a regulatory sequence isolated from a different gene or a novel regulatory sequence synthesized by genetic engineering methods. Such regulatory sequences may be comprised of promoters, enhancers, scaffold-attachment 25 regions, negative regulatory elements, transcriptional initiation sites, regulatory protein binding sites or combinations of said sequences. Alternatively, sequences which affect the structure or stability of the RNA or protein produced may be replaced, removed, added, or otherwise modified by targeting. These sequence include polyadenylation signals, mRNA stability elements, splice sites, leader sequences for enhancing or

modifying transport or secretion properties of the protein, or other sequences which alter or improve the function or stability of protein or RNA molecules.

The targeting event may be a simple insertion of the regulatory sequence, placing the gene under the control of the new regulatory sequence, *e.g.*, inserting a new 5 promoter or enhancer or both upstream of a gene. Alternatively, the targeting event may be a simple deletion of a regulatory element, such as the deletion of a tissue-specific negative regulatory element. Alternatively, the targeting event may replace an existing element; for example, a tissue-specific enhancer can be replaced by an 10 enhancer that has broader or different cell-type specificity than the naturally occurring elements. Here, the naturally occurring sequences are deleted and new sequences are added. In all cases, the identification of the targeting event may be facilitated by the 15 use of one or more selectable marker genes that are contiguous with the targeting DNA, allowing for the selection of cells in which the exogenous DNA has integrated into the host cell genome. The identification of the targeting event may also be facilitated by the use of one or more marker genes exhibiting the property of negative selection, such 20 that the negatively selectable marker is linked to the exogenous DNA, but configured such that the negatively selectable marker flanks the targeting sequence, and such that a correct homologous recombination event with sequences in the host cell genome does not result in the stable integration of the negatively selectable marker. Markers useful for this purpose include the Herpes Simplex Virus thymidine kinase (TK) gene or the bacterial xanthine-guanine phosphoribosyl-transferase (gpt) gene.

The gene targeting or gene activation techniques which can be used in accordance with this aspect of the invention are more particularly described in U.S. Patent No. 5,272,071 to Chappel; U.S. Patent No. 5,578,461 to Sherwin et al.; 25 International Application No. PCT/US92/09627 (WO93/09222) by Selden et al.; and International Application No. PCT/US90/06436 (WO91/06667) by Skoultchi et al., each of which is incorporated by reference herein in its entirety.

#### **5.4 POLYPEPTIDES OF THE INVENTION**

The isolated polypeptides of the invention include, but are not limited to, a polypeptide comprising: the amino acid sequence set forth as any one of SEQ ID NO: 4 or 6-18 or an amino acid sequence encoded by any one of the nucleotide sequences SEQ ID NO: 1-3 or 5 or the corresponding full length or mature protein. Polypeptides 5 of the invention also include polypeptides preferably with biological or immunological activity that are encoded by: (a) a polynucleotide having any one of the nucleotide sequences set forth in the SEQ ID NO: 1-3 or 5 or (b) polynucleotides encoding any one of the amino acid sequences set forth as SEQ ID NO: 4 or 6-18 or (c) 10 polynucleotides that hybridize to the complement of the polynucleotides of either (a) or (b) under stringent hybridization conditions. The invention also provides biologically active or immunologically active variants of any of the amino acid sequences set forth as SEQ ID NO: 4 or 6-18 or the corresponding full length or mature protein; and 15 "substantial equivalents" thereof (e.g., with at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, typically at least about 95%, more typically at least about 98%, or most typically at least about 99% amino acid identity) that retain biological activity. Polypeptides encoded by allelic variants may have a similar, increased, or decreased activity compared to polypeptides comprising SEQ ID NO: 4 or 6-18.

Fragments of the proteins of the present invention which are capable of 20 exhibiting biological activity are also encompassed by the present invention. Fragments of the protein may be in linear form or they may be cyclized using known methods, for example, as described in H. U. Saragovi, et al., Bio/Technology 10, 773-778 (1992) and in R. S. McDowell, et al., J. Amer. Chem. Soc. 114, 9245-9253 (1992), both of 25 which are incorporated herein by reference. Such fragments may be fused to carrier molecules such as immunoglobulins for many purposes, including increasing the valency of protein binding sites.

The present invention also provides both full-length and mature forms (for example, without a signal sequence or precursor sequence) of the disclosed proteins. 30 The protein coding sequence is identified in the sequence listing by translation of the disclosed nucleotide sequences. The mature form of such protein may be obtained by

expression of a full-length polynucleotide in a suitable mammalian cell or other host cell. The sequence of the mature form of the protein is also determinable from the amino acid sequence of the full-length form. Where proteins of the present invention are membrane bound, soluble forms of the proteins are also provided. In such forms, 5 part or all of the regions causing the proteins to be membrane bound are deleted so that the proteins are fully secreted from the cell in which it is expressed.

Protein compositions of the present invention may further comprise an acceptable carrier, such as a hydrophilic, *e.g.*, pharmaceutically acceptable, carrier.

The present invention further provides isolated polypeptides encoded by the 10 nucleic acid fragments of the present invention or by degenerate variants of the nucleic acid fragments of the present invention. By "degenerate variant" is intended nucleotide fragments which differ from a nucleic acid fragment of the present invention (*e.g.*, an ORF) by nucleotide sequence but, due to the degeneracy of the genetic code, encode an identical polypeptide sequence. Preferred nucleic acid fragments of the present 15 invention are the ORFs that encode proteins.

A variety of methodologies known in the art can be utilized to obtain any one of the isolated polypeptides or proteins of the present invention. At the simplest level, the amino acid sequence can be synthesized using commercially available peptide synthesizers. The synthetically-constructed protein sequences, by virtue of sharing 20 primary, secondary or tertiary structural and/or conformational characteristics with proteins may possess biological properties in common therewith, including protein activity. This technique is particularly useful in producing small peptides and fragments of larger polypeptides. Fragments are useful, for example, in generating antibodies against the native polypeptide. Thus, they may be employed as biologically 25 active or immunological substitutes for natural, purified proteins in screening of therapeutic compounds and in immunological processes for the development of antibodies.

The polypeptides and proteins of the present invention can alternatively be purified from cells which have been altered to express the desired polypeptide or 30 protein. As used herein, a cell is said to be altered to express a desired polypeptide or

protein when the cell, through genetic manipulation, is made to produce a polypeptide or protein which it normally does not produce or which the cell normally produces at a lower level. One skilled in the art can readily adapt procedures for introducing and expressing either recombinant or synthetic sequences into eukaryotic or prokaryotic 5 cells in order to generate a cell which produces one of the polypeptides or proteins of the present invention.

The invention also relates to methods for producing a polypeptide comprising growing a culture of host cells of the invention in a suitable culture medium, and purifying the protein from the cells or the culture in which the cells are grown. For 10 example, the methods of the invention include a process for producing a polypeptide in which a host cell containing a suitable expression vector that includes a polynucleotide of the invention is cultured under conditions that allow expression of the encoded polypeptide. The polypeptide can be recovered from the culture, conveniently from the culture medium, or from a lysate prepared from the host cells and further purified. 15 Preferred embodiments include those in which the protein produced by such process is a full length or mature form of the protein.

In an alternative method, the polypeptide or protein is purified from bacterial cells which naturally produce the polypeptide or protein. One skilled in the art can readily follow known methods for isolating polypeptides and proteins in order to obtain 20 one of the isolated polypeptides or proteins of the present invention. These include, but are not limited to, immunochromatography, HPLC, size-exclusion chromatography, ion-exchange chromatography, and immuno-affinity chromatography. See, e.g., Scopes, *Protein Purification: Principles and Practice*, Springer-Verlag (1994); Sambrook, et al., in *Molecular Cloning: A Laboratory Manual*; Ausubel et al., 25 *Current Protocols in Molecular Biology*. Polypeptide fragments that retain biological/immunological activity include fragments comprising greater than about 100 amino acids, or greater than about 200 amino acids, and fragments that encode specific protein domains.

The purified polypeptides can be used in *in vitro* binding assays which are well 30 known in the art to identify molecules which bind to the polypeptides. These molecules

include but are not limited to, for e.g., small molecules, molecules from combinatorial libraries, antibodies or other proteins. The molecules identified in the binding assay are then tested for antagonist or agonist activity in *in vivo* tissue culture or animal models that are well known in the art. In brief, the molecules are titrated into a plurality of 5 cell cultures or animals and then tested for either cell/animal death or prolonged survival of the animal/cells.

In addition, the peptides of the invention or molecules capable of binding to the peptides may be complexed with toxins, e.g., ricin or cholera, or with other compounds that are toxic to cells. The toxin-binding molecule complex is then targeted to a tumor 10 or other cell by the specificity of the binding molecule for SEQ ID NO: 4 or 6-18.

The protein of the invention may also be expressed as a product of transgenic animals, *e.g.*, as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a nucleotide sequence 15 encoding the protein.

15 The proteins provided herein also include proteins characterized by amino acid sequences similar to those of purified proteins but into which modification are naturally provided or deliberately engineered. For example, modifications, in the peptide or DNA sequence, can be made by those skilled in the art using known techniques. Modifications of interest in the protein sequences may include the alteration, 20 substitution, replacement, insertion or deletion of a selected amino acid residue in the coding sequence. For example, one or more of the cysteine residues may be deleted or replaced with another amino acid to alter the conformation of the molecule. Techniques for such alteration, substitution, replacement, insertion or deletion are well known to those skilled in the art (see, *e.g.*, U.S. Pat. No. 4,518,584). Preferably, such 25 alteration, substitution, replacement, insertion or deletion retains the desired activity of the protein. Regions of the protein that are important for the protein function can be determined by various methods known in the art including the alanine-scanning method which involved systematic substitution of single or strings of amino acids with alanine, followed by testing the resulting alanine-containing variant for biological activity. This 30 type of analysis determines the importance of the substituted amino acid(s) in biological

activity. Regions of the protein that are important for protein function may be determined by the eMATRIX program.

Other fragments and derivatives of the sequences of proteins which would be expected to retain protein activity in whole or in part and are useful for screening or  
5 other immunological methodologies may also be easily made by those skilled in the art given the disclosures herein. Such modifications are encompassed by the present invention.

The protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect  
10 expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *e.g.*, Invitrogen, San Diego, Calif., U.S.A. (the MaxBat™ kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. As  
15 used herein, an insect cell capable of expressing a polynucleotide of the present invention is "transformed."

The protein of the invention may be prepared by culturing transformed host cells under culture conditions suitable to express the recombinant protein. The resulting expressed protein may then be purified from such culture (*i.e.*, from culture medium or  
20 cell extracts) using known purification processes, such as gel filtration and ion exchange chromatography. The purification of the protein may also include an affinity column containing agents which will bind to the protein; one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl™ or Cibacrom blue 3GA Sepharose™; one or more steps involving hydrophobic interaction chromatography  
25 using such resins as phenyl ether, butyl ether, or propyl ether; or immunoaffinity chromatography.

Alternatively, the protein of the invention may also be expressed in a form which will facilitate purification. For example, it may be expressed as a fusion protein, such as those of maltose binding protein (MBP), glutathione-S-transferase (GST) or  
30 thioredoxin (TRX), or as a His tag. Kits for expression and purification of such fusion

proteins are commercially available from New England BioLab (Beverly, Mass.),  
Pharmacia (Piscataway, N.J.) and Invitrogen, respectively. The protein can also be  
tagged with an epitope and subsequently purified by using a specific antibody directed  
to such epitope. One such epitope ("FLAG®") is commercially available from Kodak  
5 (New Haven, Conn.).

Finally, one or more reverse-phase high performance liquid chromatography  
(RP-HPLC) steps employing hydrophobic RP-HPLC media, *e.g.*, silica gel having  
pendant methyl or other aliphatic groups, can be employed to further purify the protein.  
Some or all of the foregoing purification steps, in various combinations, can also be  
10 employed to provide a substantially homogeneous isolated recombinant protein. The  
protein thus purified is substantially free of other mammalian proteins and is defined in  
accordance with the present invention as an "isolated protein."

The polypeptides of the invention include analogs (variants). The polypeptides  
of the invention include LRR protein-like analogs. This embraces fragments of LRR  
15 protein-like polypeptide of the invention, as well LRR protein-like polypeptides which  
comprise one or more amino acids deleted, inserted, or substituted. Also, analogs of  
the LRR protein-like polypeptide of the invention embrace fusions of the LRR protein-  
like polypeptides or modifications of the LRR protein-like polypeptides, wherein the  
LRR protein-like polypeptide or analog is fused to another moiety or moieties, *e.g.*,  
20 targeting moiety or another therapeutic agent. Such analogs may exhibit improved  
properties such as activity and/or stability. Examples of moieties which may be fused  
to the LRR protein-like polypeptide or an analog include, for example, targeting  
moieties which provide for the delivery of polypeptide to neurons, *e.g.*, antibodies to  
central nervous system, or antibodies to receptor and ligands expressed on neuronal  
25 cells. Other moieties which may be fused to LRR protein-like polypeptide include  
therapeutic agents which are used for treatment, for example anti-depressant drugs or  
other medications for neurological disorders. Also, LRR protein-like polypeptides may  
be fused to neuron growth modulators, and other chemokines for targeted delivery.

#### **5.4.1 DETERMINING POLYPEPTIDE AND POLYNUCLEOTIDE IDENTITY AND SIMILARITY**

Preferred identity and/or similarity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in computer programs including, but are not limited to, the GCG program package, including GAP (Devereux, J., et al., Nucleic Acids Research 12(1):387 (1984); Genetics Computer Group, University of Wisconsin, Madison, WI), BLASTP, BLASTN, BLASTX, FASTA (Altschul, S.F. et al., J. Molec. Biol. 215:403-410 (1990), PSI-BLAST (Altschul S.F. et al., Nucleic Acids Res. vol. 25, pp. 3389-3402, 10 herein incorporated by reference), eMatrix software (Wu et al., J. Comp. Biol., vol. 6, pp. 219-235 (1999), herein incorporated by reference), eMotif software (Nevill-Manning et al, ISMB-97, vol 4, pp. 202-209, herein incorporated by reference) and the Kyte-Doolittle hydrophobicity prediction algorithm (J. Mol Biol, 157, pp. 105-31 15 (1982), incorporated herein by reference). The BLAST programs are publicly available from the National Center for Biotechnology Information (NCBI) and other sources (BLAST Manual, Altschul, S., et al. NCB NLM NIH Bethesda, MD 20894; Altschul, S., et al., J. Mol. Biol. 215:403-410 (1990).

#### **5.5 GENE THERAPY**

Mutations in the polynucleotides of the invention gene may result in loss of normal function of the encoded protein. The invention thus provides gene therapy to restore normal activity of the polypeptides of the invention; or to treat disease states involving polypeptides of the invention. Delivery of a functional gene encoding polypeptides of the invention to appropriate cells is effected *ex vivo*, *in situ*, or *in vivo* 20 by use of vectors, and more particularly viral vectors (e.g., adenovirus, adeno-associated virus, or a retrovirus), or *ex vivo* by use of physical DNA transfer methods (e.g., liposomes or chemical treatments). See, for example, Anderson, Nature, supplement to vol. 392, no. 6679, pp.25-20 (1998). For additional reviews of gene 25 therapy technology see Friedmann, Science, 244: 1275-1281 (1989); Verma, Scientific American: 68-84 (1990); and Miller, Nature, 357: 455-460 (1992). Introduction of any 30

one of the nucleotides of the present invention or a gene encoding the polypeptides of the present invention can also be accomplished with extrachromosomal substrates (transient expression) or artificial chromosomes (stable expression). Cells may also be cultured *ex vivo* in the presence of proteins of the present invention in order to 5 proliferate or to produce a desired effect on or activity in such cells. Treated cells can then be introduced *in vivo* for therapeutic purposes. Alternatively, it is contemplated that in other human disease states, preventing the expression of or inhibiting the activity of polypeptides of the invention will be useful in treating the disease states. It is contemplated that antisense therapy or gene therapy could be applied to negatively 10 regulate the expression of polypeptides of the invention.

Other methods inhibiting expression of a protein include the introduction of antisense molecules to the nucleic acids of the present invention, their complements, or their translated RNA sequences, by methods known in the art. Further, the polypeptides of the present invention can be inhibited by using targeted deletion methods, or the 15 insertion of a negative regulatory element such as a silencer, which is tissue specific.

The present invention still further provides cells genetically engineered *in vivo* to express the polynucleotides of the invention, wherein such polynucleotides are in operative association with a regulatory sequence heterologous to the host cell which drives expression of the polynucleotides in the cell. These methods can be used to increase or 20 decrease the expression of the polynucleotides of the present invention.

Knowledge of DNA sequences provided by the invention allows for modification of cells to permit, increase, or decrease, expression of endogenous polypeptide. Cells can be modified (e.g., by homologous recombination) to provide increased polypeptide expression by replacing, in whole or in part, the naturally occurring promoter with all or 25 part of a heterologous promoter so that the cells express the protein at higher levels. The heterologous promoter is inserted in such a manner that it is operatively linked to the desired protein encoding sequences. See, for example, PCT International Publication No. WO 94/12650, PCT International Publication No. WO 92/20808, and PCT International Publication No. WO 91/09955. It is also contemplated that, in addition to heterologous 30 promoter DNA, amplifiable marker DNA (e.g., ada, dhfr, and the multifunctional CAD

gene which encodes carbamyl phosphate synthase, aspartate transcarbamylase, and dihydroorotate) and/or intron DNA may be inserted along with the heterologous promoter DNA. If linked to the desired protein coding sequence, amplification of the marker DNA by standard selection methods results in co-amplification of the desired protein coding sequences in the cells.

In another embodiment of the present invention, cells and tissues may be engineered to express an endogenous gene comprising the polynucleotides of the invention under the control of inducible regulatory elements, in which case the regulatory sequences of the endogenous gene may be replaced by homologous recombination. As described herein, gene targeting can be used to replace a gene's existing regulatory region with a regulatory sequence isolated from a different gene or a novel regulatory sequence synthesized by genetic engineering methods. Such regulatory sequences may be comprised of promoters, enhancers, scaffold-attachment regions, negative regulatory elements, transcriptional initiation sites, regulatory protein binding sites or combinations of said sequences. Alternatively, sequences which affect the structure or stability of the RNA or protein produced may be replaced, removed, added, or otherwise modified by targeting. These sequences include polyadenylation signals, mRNA stability elements, splice sites, leader sequences for enhancing or modifying transport or secretion properties of the protein, or other sequences which alter or improve the function or stability of protein or RNA molecules.

The targeting event may be a simple insertion of the regulatory sequence, placing the gene under the control of the new regulatory sequence, *e.g.*, inserting a new promoter or enhancer or both upstream of a gene. Alternatively, the targeting event may be a simple deletion of a regulatory element, such as the deletion of a tissue-specific negative regulatory element. Alternatively, the targeting event may replace an existing element; for example, a tissue-specific enhancer can be replaced by an enhancer that has broader or different cell-type specificity than the naturally occurring elements. Here, the naturally occurring sequences are deleted and new sequences are added. In all cases, the identification of the targeting event may be facilitated by the use of one or more selectable marker genes that are contiguous with the targeting DNA, allowing for the selection of

cells in which the exogenous DNA has integrated into the cell genome. The identification of the targeting event may also be facilitated by the use of one or more marker genes exhibiting the property of negative selection, such that the negatively selectable marker is linked to the exogenous DNA, but configured such that the negatively selectable marker  
5 flanks the targeting sequence, and such that a correct homologous recombination event with sequences in the host cell genome does not result in the stable integration of the negatively selectable marker. Markers useful for this purpose include the Herpes Simplex Virus thymidine kinase (TK) gene or the bacterial xanthine-guanine phosphoribosyl-transferase (gpt) gene.

10 The gene targeting or gene activation techniques which can be used in accordance with this aspect of the invention are more particularly described in U.S. Patent No. 5,272,071 to Chappel; U.S. Patent No. 5,578,461 to Sherwin et al.; International Application No. PCT/US92/09627 (WO93/09222) by Selden et al.; and International Application No. PCT/US90/06436 (WO91/06667) by Skoultchi et al., each of which is  
15 incorporated by reference herein in its entirety.

## 5.6 TRANSGENIC ANIMALS

In preferred methods to determine biological functions of the polypeptides of the invention in vivo, one or more genes provided by the invention are either over expressed or inactivated in the germ line of animals using homologous recombination [Capecchi, Science 244:1288-1292 (1989)]. Animals in which the gene is over expressed, under the regulatory control of exogenous or endogenous promoter elements, are known as transgenic animals. Animals in which an endogenous gene has been inactivated by homologous recombination are referred to as "knockout" animals.  
20 Knockout animals, preferably non-human mammals, can be prepared as described in U.S. Patent No. 5,557,032, incorporated herein by reference. Transgenic animals are useful to determine the roles polypeptides of the invention play in biological processes, and preferably in disease states. Transgenic animals are useful as model systems to identify compounds that modulate lipid metabolism. Transgenic animals, preferably  
25

non-human mammals, are produced using methods as described in U.S. Patent No 5,489,743 and PCT Publication No. WO94/28122, incorporated herein by reference.

Transgenic animals can be prepared wherein all or part of a promoter of the polynucleotides of the invention is either activated or inactivated to alter the level of expression of the polypeptides of the invention. Inactivation can be carried out using homologous recombination methods described above. Activation can be achieved by supplementing or even replacing the homologous promoter to provide for increased protein expression. The homologous promoter can be supplemented by insertion of one or more heterologous enhancer elements known to confer promoter activation in a particular tissue.

The polynucleotides of the present invention also make possible the development, through, e.g., homologous recombination or knock out strategies; of animals that fail to express functional LRR protein-like polypeptide or that express a variant of LRR protein-like polypeptide. Such animals are useful as models for studying the *in vivo* activities of LRR protein-like polypeptide as well as for studying modulators of the LRR protein-like polypeptide.

In preferred methods to determine biological functions of the polypeptides of the invention *in vivo*, one or more genes provided by the invention are either over expressed or inactivated in the germ line of animals using homologous recombination [Capecci, Science 244:1288-1292 (1989)]. Animals in which the gene is over expressed, under the regulatory control of exogenous or endogenous promoter elements, are known as transgenic animals. Animals in which an endogenous gene has been inactivated by homologous recombination are referred to as "knockout" animals. Knockout animals, preferably non-human mammals, can be prepared as described in U.S. Patent No. 5,557,032, incorporated herein by reference. Transgenic animals are useful to determine the roles polypeptides of the invention play in biological processes, and preferably in disease states. Transgenic animals are useful as model systems to identify compounds that modulate lipid metabolism. Transgenic animals, preferably non-human mammals, are produced using methods as described in U.S. Patent No 5,489,743 and PCT Publication No. WO94/28122, incorporated herein by reference.

Transgenic animals can be prepared wherein all or part of the polynucleotides of the invention promoter is either activated or inactivated to alter the level of expression of the polypeptides of the invention. Inactivation can be carried out using homologous recombination methods described above. Activation can be achieved by supplementing or even replacing the homologous promoter to provide for increased protein expression. The homologous promoter can be supplemented by insertion of one or more heterologous enhancer elements known to confer promoter activation in a particular tissue.

10 **5.7 USES AND BIOLOGICAL ACTIVITY OF HUMAN LRR PROTEIN-LIKE  
POLYPEPTIDE**

The polynucleotides and proteins of the present invention are expected to exhibit one or more of the uses or biological activities (including those associated with assays cited herein) identified herein. Uses or activities described for proteins of the present invention may be provided by administration or use of such proteins or of polynucleotides encoding such proteins (such as, for example, in gene therapies or vectors suitable for introduction of DNA). The mechanism underlying the particular condition or pathology will dictate whether the polypeptides of the invention, the polynucleotides of the invention or modulators (activators or inhibitors) thereof would be beneficial to the subject in need of treatment. Thus, "therapeutic compositions of the invention" include compositions comprising isolated polynucleotides (including recombinant DNA molecules, cloned genes and degenerate variants thereof) or polypeptides of the invention (including full length protein, mature protein and truncations or domains thereof), or compounds and other substances that modulate the overall activity of the target gene products, either at the level of target gene/protein expression or target protein activity. Such modulators include polypeptides, analogs, (variants), including fragments and fusion proteins, antibodies and other binding proteins; chemical compounds that directly or indirectly activate or inhibit the polypeptides of the invention (identified, e.g., via drug screening assays as described herein); antisense polynucleotides and polynucleotides suitable for triple helix

formation; and in particular antibodies or other binding partners that specifically recognize one or more epitopes of the polypeptides of the invention.

The polypeptides of the present invention may likewise be involved in cellular activation or in one of the other physiological pathways described herein.

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### **5.7.1 RESEARCH USES AND UTILITIES**

The polynucleotides provided by the present invention can be used by the research community for various purposes. The polynucleotides can be used to express recombinant protein for analysis, characterization or therapeutic use; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in disease states); as molecular weight markers on gels; as chromosome markers or tags (when labeled) to identify chromosomes or to map related gene positions; to compare with endogenous DNA sequences in patients to identify potential genetic disorders; as probes to hybridize and thus discover novel, related DNA sequences; as a source of information to derive PCR primers for genetic fingerprinting; as a probe to "subtract-out" known sequences in the process of discovering other novel polynucleotides; for selecting and making oligomers for attachment to a "gene chip" or other support, including for examination of expression patterns; to raise anti-protein antibodies using DNA immunization techniques; and as an antigen to raise anti-DNA antibodies or elicit another immune response. Where the polynucleotide encodes a protein which binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the polynucleotide can also be used in interaction trap assays (such as, for example, that described in Gyuris et al., Cell 75:791-803 (1993)) to identify polynucleotides encoding the other protein with which binding occurs or to identify inhibitors of the binding interaction.

The polypeptides provided by the present invention can similarly be used in assays to determine biological activity, including in a panel of multiple proteins for high-throughput screening; to raise antibodies or to elicit another immune response; as a reagent (including the labeled reagent) in assays designed to quantitatively determine

levels of the protein (or its receptor) in biological fluids; as markers for tissues in which the corresponding polypeptide is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in a disease state); and, of course, to isolate correlative receptors or ligands. Proteins involved in these binding  
5 interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction.

The polypeptides of the invention are also useful for making antibody substances that are specifically immunoreactive with LRR protein-like proteins. Antibodies and portions thereof (e.g., Fab fragments) which bind to the polypeptides of the invention  
10 can be used to identify the presence of such polypeptides in a sample. Such determinations are carried out using any suitable immunoassay format, and any polypeptide of the invention that is specifically bound by the antibody can be employed as a positive control.

Any or all of these research utilities are capable of being developed into reagent  
15 grade or kit format for commercialization as research products.

Methods for performing the uses listed above are well known to those skilled in the art. References disclosing such methods include without limitation "Molecular Cloning: A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory Press, Sambrook, J., E. F. Fritsch and T. Maniatis eds., 1989, and "Methods in Enzymology:  
20 Guide to Molecular Cloning Techniques", Academic Press, Berger, S. L. and A. R. Kimmel eds., 1987.

### **5.7.2 NUTRITIONAL USES**

Polynucleotides and polypeptides of the present invention can also be used as  
25 nutritional sources or supplements. Such uses include without limitation use as a protein or amino acid supplement, use as a carbon source, use as a nitrogen source and use as a source of carbohydrate. In such cases the polypeptide or polynucleotide of the invention can be added to the feed of a particular organism or can be administered as a separate solid or liquid preparation, such as in the form of powder, pills, solutions, suspensions or

capsules. In the case of microorganisms, the polypeptide or polynucleotide of the invention can be added to the medium in or on which the microorganism is cultured.

Additionally, the polypeptides of the invention can be used as molecular weight markers, and as a food supplement. A polypeptide consisting of SEQ ID NO: 4, for example, has a molecular mass of approximately 77.5 kDa in its unprocessed and unglycosylated state. Protein food supplements are well known and the formulation of suitable food supplements including polypeptides of the invention is within the level of skill in the food preparation art.

10           **5.7.3 CYTOKINE AND CELL PROLIFERATION/DIFFERENTIATION**  
ACTIVITY

A polypeptide of the present invention may exhibit activity relating to cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. A polynucleotide of the invention can encode a polypeptide exhibiting such attributes. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor-dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of therapeutic compositions of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+(preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e, CMK, HUVEC, and Caco. Therapeutic compositions of the invention can be used in the following:

Assays for T-cell or thymocyte proliferation include without limitation those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, *In Vitro* assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Bertagnoli et al., J. Immunol. 145:1706-1712, 1990;

Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Bertagnolli, et al., I. Immunol. 149:3778-3783, 1992; Bowman et al., I. Immunol. 152:1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Polyclonal T cell stimulation, Kruisbeek, A. M. and Shevach, E. M. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons, Toronto. 1994; and Measurement of mouse and human interleukin- $\gamma$ , Schreiber, R. D. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto. 1994.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described in: Measurement of Human and Murine Interleukin 2 and Interleukin 4, Bottomly, K., Davis, L. S. and Lipsky, P. E. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto. 1991; deVries et al., J. Exp. Med. 173:1205-1211, 1991; Moreau et al., Nature 336:690-692, 1988; Greenberger et al., Proc. Natl. Acad. Sci. U.S.A. 80:2931-2938, 1983; Measurement of mouse and human interleukin 6--Nordan, R. In Current Protocols in Immunology. J. E. Coligan eds. Vol 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto. 1991; Smith et al., Proc. Natl. Acad. Sci. U.S.A. 83:1857-1861, 1986; Measurement of human Interleukin 11--Bennett, F.,

Giannotti, J., Clark, S. C. and Turner, K. J. In Current Protocols in Immunology. J. E. Coligan eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto. 1991; Measurement of mouse and human Interleukin 9-Ciarletta, A., Giannotti, J., Clark, S. C. and Turner, K. J. In Current Protocols in Immunology. J. E. Coligan eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto. 1991.

Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, *In Vitro* assays for Mouse Lymphocyte

Function; Chapter 6, Cytokines and their cellular receptors; Chapter 7, Immunologic studies in Humans); Weinberger et al., Proc. Natl. Acad. Sci. USA 77:6091-6095, 1980; Weinberger et al., Eur. J. Immun. 11:405-411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988.

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#### **5.7.4 STEM CELL GROWTH FACTOR ACTIVITY**

A polypeptide of the present invention may exhibit stem cell growth factor activity and be involved in the proliferation, differentiation and survival of pluripotent and totipotent stem cells including primordial germ cells, embryonic stem cells, hematopoietic stem cells and/or germ line stem cells. Administration of the polypeptide of the invention to stem cells *in vivo* or *ex vivo* may maintain and expand cell populations in a totipotential or pluripotential state which would be useful for re-engineering damaged or diseased tissues, transplantation, manufacture of bio-pharmaceuticals and the development of bio-sensors. The ability to produce large quantities of human cells has important working applications for the production of human proteins which currently must be obtained from non-human sources or donors, implantation of cells to treat diseases such as Parkinson's, Alzheimer's and other neurodegenerative diseases; tissues for grafting such as bone marrow, skin, cartilage, tendons, bone, muscle (including cardiac muscle), blood vessels, cornea, neural cells, gastrointestinal cells and others; and organs for transplantation such as kidney, liver, pancreas (including islet cells), heart and lung.

It is contemplated that multiple different exogenous growth factors and/or cytokines may be administered in combination with the polypeptide of the invention to achieve the desired effect, including any of the growth factors listed herein, other stem cell maintenance factors, and specifically including stem cell factor (SCF), leukemia inhibitory factor (LIF), Flt-3 ligand (Flt-3L), any of the interleukins, recombinant soluble IL-6 receptor fused to IL-6, macrophage inflammatory protein 1-alpha (MIP-1 alpha), G-CSF, GM-CSF, thrombopoietin (TPO), platelet factor 4 (PF-4), platelet-derived growth factor (PDGF), neural growth factors and basic fibroblast growth factor (bFGF).

Since totipotent stem cells can give rise to virtually any mature cell type, expansion of these cells in culture will facilitate the production of large quantities of mature cells. Techniques for culturing stem cells are known in the art and administration of polypeptides of the invention, optionally with other growth factors and/or cytokines, is expected to enhance the survival and proliferation of the stem cell populations. This can be accomplished by direct administration of the polypeptide of the invention to the culture medium. Alternatively, stroma cells transfected with a polynucleotide that encodes for the polypeptide of the invention can be used as a feeder layer for the stem cell populations in culture or in vivo. Stromal support cells for feeder layers may include embryonic bone marrow fibroblasts, bone marrow stromal cells, fetal liver cells, or cultured embryonic fibroblasts (see U.S. Patent No. 5,690,926).

Stem cells themselves can be transfected with a polynucleotide of the invention to induce autocrine expression of the polypeptide of the invention. This will allow for generation of undifferentiated totipotential/pluripotential stem cell lines that are useful as is or that can then be differentiated into the desired mature cell types. These stable cell lines can also serve as a source of undifferentiated totipotential/pluripotential mRNA to create cDNA libraries and templates for polymerase chain reaction experiments. These studies would allow for the isolation and identification of differentially expressed genes in stem cell populations that regulate stem cell proliferation and/or maintenance.

Expansion and maintenance of totipotent stem cell populations will be useful in the treatment of many pathological conditions. For example, polypeptides of the present invention may be used to manipulate stem cells in culture to give rise to neuroepithelial cells that can be used to augment or replace cells damaged by illness, autoimmune disease, accidental damage or genetic disorders. The polypeptide of the invention may be useful for inducing the proliferation of neural cells and for the regeneration of nerve and brain tissue, i.e. for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders which involve degeneration, death or trauma to neural cells or nerve tissue. In

addition, the expanded stem cell populations can also be genetically altered for gene therapy purposes and to decrease host rejection of replacement tissues after grafting or implantation.

Expression of the polypeptide of the invention and its effect on stem cells can 5 also be manipulated to achieve controlled differentiation of the stem cells into more differentiated cell types. A broadly applicable method of obtaining pure populations of a specific differentiated cell type from undifferentiated stem cell populations involves the use of a cell-type specific promoter driving a selectable marker. The selectable marker allows only cells of the desired type to survive. For example, stem cells can be 10 induced to differentiate into cardiomyocytes (Wobus et al., Differentiation, 48: 173-182, (1991); Klug et al., J. Clin. Invest., 98(1): 216-224, (1998)) or skeletal muscle cells (Browder, L. W. In: *Principles of Tissue Engineering* eds. Lanza et al., Academic Press (1997)). Alternatively, directed differentiation of stem cells can be 15 accomplished by culturing the stem cells in the presence of a differentiation factor such as retinoic acid and an antagonist of the polypeptide of the invention which would inhibit the effects of endogenous stem cell factor activity and allow differentiation to proceed.

*In vitro* cultures of stem cells can be used to determine if the polypeptide of the invention exhibits stem cell growth factor activity. Stem cells are isolated from any one 20 of various cell sources (including hematopoietic stem cells and embryonic stem cells) and cultured on a feeder layer, as described by Thompson et al. Proc. Natl. Acad. Sci., U.S.A., 92: 7844-7848 (1995), in the presence of the polypeptide of the invention alone or in combination with other growth factors or cytokines. The ability of the 25 polypeptide of the invention to induce stem cells proliferation is determined by colony formation on semi-solid support e.g. as described by Bernstein et al., Blood, 77: 2316-2321 (1991).

### 5.7.5 HEMATOPOIESIS REGULATING ACTIVITY

A polypeptide of the present invention may be involved in regulation of 30 hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell

disorders. Even marginal biological activity in support of colony forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis, e.g. in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating

5 various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (i.e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and

10 proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post

15 irradiation/chemotherapy, either *in-vivo* or *ex-vivo* (i.e., in conjunction with bone marrow transplantation or with peripheral progenitor cell transplantation (homologous or heterologous)) as normal cells or genetically manipulated for gene therapy.

Therapeutic compositions of the invention can be used in the following:

Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

Assays for embryonic stem cell differentiation (which will identify, among

25 others, proteins that influence embryonic differentiation hematopoiesis) include, without limitation, those described in: Johansson et al. Cellular Biology 15:141-151, 1995; Keller et al., Molecular and Cellular Biology 13:473-486, 1993; McClanahan et al., Blood 81:2903-2915, 1993.

Assays for stem cell survival and differentiation (which will identify, among

30 others, proteins that regulate lympho-hematopoiesis) include, without limitation, those

described in: Methylcellulose colony forming assays, Freshney, M. G. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 265-268, Wiley-Liss, Inc., New York, N.Y. 1994; Hirayama et al., Proc. Natl. Acad. Sci. USA 89:5907-5911, 1992; Primitive hematopoietic colony forming cells with high proliferative potential,  
5 McNiece, I. K. and Briddell, R. A. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, N.Y. 1994; Neben et al., Experimental Hematology 22:353-359, 1994; Cobblestone area forming cell assay, Ploemacher, R. E. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 1-21, Wiley-Liss, Inc., New York, N.Y. 1994; Long term bone marrow cultures in  
10 the presence of stromal cells, Spooncer, E., Dexter, M. and Allen, T. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 163-179, Wiley-Liss, Inc., New York, N.Y. 1994; Long term culture initiating cell assay, Sutherland, H. J. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 139-162, Wiley-Liss, Inc., New York, N.Y. 1994.

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### **5.7.6 TISSUE GROWTH ACTIVITY**

A polypeptide of the present invention also may be involved in bone, cartilage, tendon, ligament and/or nerve tissue growth or regeneration, as well as in wound healing and tissue repair and replacement, and in healing of burns, incisions and ulcers.

20

A polypeptide of the present invention which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Compositions of a polypeptide, antibody, binding partner, or other modulator of the invention may have prophylactic use in closed as well as open fracture reduction and  
25 also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

30

A polypeptide of this invention may also be involved in attracting bone-forming cells, stimulating growth of bone-forming cells, or inducing differentiation of progenitors of bone-forming cells. Treatment of osteoporosis, osteoarthritis, bone

degenerative disorders, or periodontal disease, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes may also be possible using the composition of the invention.

5 Another category of tissue regeneration activity that may involve the polypeptide of the present invention is tendon/ligament formation. Induction of tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears, deformities and other tendon or ligament defects in humans and other animals. Such a preparation employing  
10 a tendon/ligament-like tissue inducing protein may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to tendon or ligament tissue. *De novo* tendon/ligament-like tissue formation induced by a composition of the present invention contributes to the repair of congenital, trauma induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery  
15 for attachment or repair of tendons or ligaments. The compositions of the present invention may provide environment to attract tendon- or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon/ligament  
20 cells or progenitors *ex vivo* for return *in vivo* to effect tissue repair. The compositions of the invention may also be useful in the treatment of tendinitis, carpal tunnel syndrome and other tendon or ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

The compositions of the present invention may also be useful for proliferation of  
25 neural cells and for regeneration of nerve and brain tissue, i.e. for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders, which involve degeneration, death or trauma to neural cells or nerve tissue. More specifically, a composition may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral  
30 neuropathy and localized neuropathies, and central nervous system diseases, such as

Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions which may be treated in accordance with the present invention include mechanical and traumatic disorders, such as spinal cord disorders, head trauma and cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using a composition of the invention.

Compositions of the invention may also be useful to promote better or faster closure of non-healing wounds, including without limitation pressure ulcers, ulcers associated with vascular insufficiency, surgical and traumatic wounds, and the like.

Compositions of the present invention may also be involved in the generation or regeneration of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac) and vascular (including vascular endothelium) tissue, or for promoting the growth of cells comprising such tissues. Part of the desired effects may be by inhibition or modulation of fibrotic scarring may allow normal tissue to regenerate. A polypeptide of the present invention may also exhibit angiogenic activity.

A composition of the present invention may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage.

A composition of the present invention may also be useful for promoting or inhibiting differentiation of tissues described above from precursor tissues or cells; or for inhibiting the growth of tissues described above.

Therapeutic compositions of the invention can be used in the following:

Assays for tissue generation activity include, without limitation, those described in: International Patent Publication No. WO95/16035 (bone, cartilage, tendon); International Patent Publication No. WO95/05846 (nerve, neuronal); International Patent Publication No. WO91/07491 (skin, endothelium).

Assays for wound healing activity include, without limitation, those described in: Winter, Epidermal Wound Healing, pp. 71-112 (Maibach, H. I. and Rovee, D. T.,

eds.), Year Book Medical Publishers, Inc., Chicago, as modified by Eaglstein and Mertz, J. Invest. Dermatol 71:382-84 (1978).

#### **5.7.7 IMMUNE FUNCTION STIMULATING OR SUPPRESSING**

##### **5 ACTIVITY**

A polypeptide of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A polynucleotide of the invention can encode a polypeptide exhibiting such activities. A protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases causes by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpes viruses, mycobacteria, Leishmania spp., malaria spp. and various fungal infections such as candidiasis. Of course, in this regard, proteins of the present invention may also be useful where a boost to the immune system generally may be desirable, i.e., in the treatment of cancer.

Autoimmune disorders which may be treated using a protein of the present invention include, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitus, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein (or antagonists thereof, including antibodies) of the present invention may also be useful in the treatment of allergic reactions and conditions (e.g., anaphylaxis, serum sickness, drug reactions, food allergies, insect venom allergies, mastocytosis, allergic rhinitis, hypersensitivity pneumonitis, urticaria, angioedema, eczema, atopic dermatitis, allergic contact dermatitis, erythema multiforme, Stevens-

Johnson syndrome, allergic conjunctivitis, atopic keratoconjunctivitis, venereal keratoconjunctivitis, giant papillary conjunctivitis and contact allergies), such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, organ transplantation), may

5 also be treatable using a protein (or antagonists thereof) of the present invention. The therapeutic effects of the polypeptides or antagonists thereof on allergic reactions can be evaluated by in vivo animals models such as the cumulative contact enhancement test (Lastbom et al., Toxicology 125: 59-66, 1998), skin prick test (Hoffmann et al., Allergy 54: 446-54, 1999), guinea pig skin sensitization test (Vohr et al., Arch. 10 Toxocol. 73: 501-9), and murine local lymph node assay (Kimber et al., J. Toxicol. Environ. Health 53: 563-79).

Using the proteins of the invention it may also be possible to modulate immune responses, in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing the

15 induction of an immune response. The functions of activated T cells may be inhibited by suppressing T cell responses or by inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is generally an active, non-antigen-specific, process which requires continuous exposure of the T cells to the suppressive agent. Tolerance, which involves inducing non-responsiveness or anergy in T cells, is 20 distinguishable from immunosuppression in that it is generally antigen-specific and persists after exposure to the tolerizing agent has ceased. Operationally, tolerance can be demonstrated by the lack of a T cell response upon reexposure to specific antigen in the absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions (such as, for example, B7)), e.g., preventing high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated

30 through its recognition as foreign by T cells, followed by an immune reaction that

destroys the transplant. The administration of a therapeutic composition of the invention may prevent cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, a lack of costimulation may also be sufficient to anergize the T cells, thereby inducing tolerance in a subject. Induction of long-term  
5 tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or tolerance in a subject, it may also be necessary to block the function of a combination of B lymphocyte antigens.

The efficacy of particular therapeutic compositions in preventing organ  
10 transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Ig fusion proteins in vivo as described in Lenschow et al., Science 257:789-792 (1992) and  
15 Turka et al., Proc. Natl. Acad. Sci USA, 89:11102-11105 (1992). In addition, murine models of GVHD (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of therapeutic compositions of the invention on the development of that disease.

Blocking antigen function may also be therapeutically useful for treating  
20 autoimmune diseases. Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self tissue and which promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms. Administration of reagents which block stimulation of T cells can be used to  
25 inhibit T cell activation and prevent production of autoantibodies or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of autoreactive T cells which could lead to long-term relief from the disease. The efficacy of blocking reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine  
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experimental autoimmune encephalitis, systemic lupus erythematosus in MRL/lpr/lpr mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 840-856).

5 Upregulation of an antigen function (e.g., a B lymphocyte antigen function), as a means of up regulating immune responses, may also be useful in therapy.

Upregulation of immune responses may be in the form of enhancing an existing immune response or eliciting an initial immune response. For example, enhancing an immune response may be useful in cases of viral infection, including systemic viral 10 diseases such as influenza, the common cold, and encephalitis.

Alternatively, anti-viral immune responses may be enhanced in an infected patient by removing T cells from the patient, costimulating the T cells in vitro with viral antigen-pulsed APCs either expressing a peptide of the present invention or together with a stimulatory form of a soluble peptide of the present invention and 15 reintroducing the in vitro activated T cells into the patient. Another method of enhancing anti-viral immune responses would be to isolate infected cells from a patient, transfect them with a nucleic acid encoding a protein of the present invention as described herein such that the cells express all or a portion of the protein on their surface, and reintroduce the transfected cells into the patient. The infected cells would 20 now be capable of delivering a costimulatory signal to, and thereby activate, T cells in vivo.

A polypeptide of the present invention may provide the necessary stimulation signal to T cells to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor cells which lack MHC class I or MHC class II 25 molecules, or which fail to reexpress sufficient mounts of MHC class I or MHC class II molecules, can be transfected with nucleic acid encoding all or a portion of (e.g., a cytoplasmic-domain truncated portion) of an MHC class I alpha chain protein and  $\beta_2$  microglobulin protein or an MHC class II alpha chain protein and an MHC class II beta chain protein to thereby express MHC class I or MHC class II proteins on the cell 30 surface. Expression of the appropriate class I or class II MHC in conjunction with a

peptide having the activity of a B lymphocyte antigen (e.g., B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as the invariant chain, can also be cotransfected with a DNA 5 encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated immune response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject.

The activity of a protein of the invention may, among other means, be measured 10 by the following methods:

Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse 15 Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., I. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Bowman et al., J. Virology 61:1992-1998; Bertagnolli et al., Cellular 20 Immunology 133:327-341, 1991; Brown et al., J. Immunol. 153:3079-3092, 1994.

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J. Immunol. 144:3028-3033, 1990; and Assays for B cell function: In 25 vitro antibody production, Mond, J. J. and Brunswick, M. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto. 1994.

Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without 30 limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan,

A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; 5 Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, proteins expressed by dendritic cells that activate naive T-cells) include, without limitation, those described in: Guery et al., J. Immunol. 134:536-544, 1995; Inaba et al., Journal of Experimental Medicine 173:549-559, 1991; Macatonia et al., Journal of

10 Immunology 154:5071-5079, 1995; Porgador et al., Journal of Experimental Medicine 182:255-260, 1995; Nair et al., Journal of Virology 67:4062-4069, 1993; Huang et al., Science 264:961-965, 1994; Macatonia et al., Journal of Experimental Medicine 169:1255-1264, 1989; Bhardwaj et al., Journal of Clinical Investigation 94:797-807, 1994; and Inaba et al., Journal of Experimental Medicine 172:631-640, 1990.

15 Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz et al., Cytometry 13:795-808, 1992; Gorczyca et al., Leukemia 7:659-670, 1993; Gorczyca et al., Cancer Research 53:1945-1951, 1993; Itoh et al., Cell 66:233-243, 20 1991; Zacharchuk, Journal of Immunology 145:4037-4045, 1990; Zamai et al., Cytometry 14:891-897, 1993; Gorczyca et al., International Journal of Oncology 1:639-648, 1992.

25 Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica et al., Blood 84:111-117, 1994; Fine et al., Cellular Immunology 155:111-122, 1994; Galy et al., Blood 85:2770-2778, 1995; Toki et al., Proc. Nat. Acad Sci. USA 88:7548-7551, 1991.

### 5.7.8 ACTIVIN/INHIBIN ACTIVITY

A polypeptide of the present invention may also exhibit activin- or inhibin-related activities. A polynucleotide of the invention may encode a polypeptide exhibiting such characteristics. Inhibins are characterized by their ability to inhibit the release of follicle stimulating hormone (FSH), while activins are characterized by their ability to stimulate the release of follicle stimulating hormone (FSH). Thus, a polypeptide of the present invention, alone or in heterodimers with a member of the inhibin family, may be useful as a contraceptive based on the ability of inhibins to decrease fertility in female mammals and decrease spermatogenesis in male mammals.

5 Administration of sufficient amounts of other inhibins can induce infertility in these mammals. Alternatively, the polypeptide of the invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin group, may be useful as a fertility inducing therapeutic, based upon the ability of activin molecules in stimulating FSH release from cells of the anterior pituitary. See, for example, U.S. Pat. No. 4,798,885.

10 A polypeptide of the invention may also be useful for advancement of the onset of fertility in sexually immature mammals, so as to increase the lifetime reproductive performance of domestic animals such as, but not limited to, cows, sheep and pigs.

15

The activity of a polypeptide of the invention may, among other means, be measured by the following methods.

Assays for activin/inhibin activity include, without limitation, those described  
20 in: Vale et al., Endocrinology 91:562-572, 1972; Ling et al., Nature 321:779-782,  
1986; Vale et al., Nature 321:776-779, 1986; Mason et al., Nature 318:659-663, 1985;  
Forage et al., Proc. Natl. Acad. Sci. USA 83:3091-3095, 1986.

### 5.7.9 CHEMOTACTIC/CHEMOKINETIC ACTIVITY

25 A polypeptide of the present invention may be involved in chemotactic or chemokinetic activity for mammalian cells, including, for example, monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells. A polynucleotide of the invention can encode a polypeptide exhibiting such attributes. Chemotactic and chemokinetic receptor activation can be used to mobilize  
30 or attract a desired cell population to a desired site of action. Chemotactic or

chemokinetic compositions (e.g. proteins, antibodies, binding partners, or modulators of the invention) provide particular advantages in treatment of wounds and other trauma to tissues, as well as in treatment of localized infections. For example, attraction of lymphocytes, monocytes or neutrophils to tumors or sites of infection may result in  
5 improved immune responses against the tumor or infecting agent.

A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such cell population. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a  
10 population of cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

Therapeutic compositions of the invention can be used in the following:

Assays for chemotactic activity (which will identify proteins that induce or prevent chemotaxis) consist of assays that measure the ability of a protein to induce the migration of cells across a membrane as well as the ability of a protein to induce the adhesion of one cell population to another cell population. Suitable assays for movement and adhesion include, without limitation, those described in: Current  
15 Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Marguiles, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience  
20 (Chapter 6.12, Measurement of alpha and beta Chemokines 6.12.1-6.12.28; Taub et al. J. Clin. Invest. 95:1370-1376, 1995; Lind et al. APMIS 103:140-146, 1995; Muller et al Eur. J. Immunol. 25:1744-1748; Gruber et al. J. of Immunol. 152:5860-5867, 1994; Johnston et al. J. of Immunol. 153:1762-1768, 1994.

#### 25           **5.7.10 HEMOSTATIC AND THROMBOLYTIC ACTIVITY**

A polypeptide of the invention may also be involved in hemostasis or thrombolysis or thrombosis. A polynucleotide of the invention can encode a polypeptide exhibiting such attributes. Compositions may be useful in treatment of various coagulation disorders (including hereditary disorders, such as hemophilias) or  
30 to enhance coagulation and other hemostatic events in treating wounds resulting from

trauma, surgery or other causes. A composition of the invention may also be useful for dissolving or inhibiting formation of thromboses and for treatment and prevention of conditions resulting therefrom (such as, for example, infarction of cardiac and central nervous system vessels (e.g., stroke).

5 Therapeutic compositions of the invention can be used in the following:

Assay for hemostatic and thromolytic activity include, without limitation, those described in: Linet et al., J. Clin. Pharmacol. 26:131-140, 1986; Burdick et al., Thrombosis Res. 45:413-419, 1987; Humphrey et al., Fibrinolysis 5:71-79 (1991); Schaub, Prostaglandins 35:467-474, 1988.

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### **5.7.11 CANCER DIAGNOSIS AND THERAPY**

Polypeptides of the invention may be involved in cancer cell generation, proliferation or metastasis. Detection of the presence or amount of polynucleotides or polypeptides of the invention may be useful for the diagnosis and/or prognosis of one or 15 more types of cancer. For example, the presence or increased expression of a polynucleotide/polypeptide of the invention may indicate a hereditary risk of cancer, a precancerous condition, or an ongoing malignancy. Conversely, a defect in the gene or absence of the polypeptide may be associated with a cancer condition. Identification of single nucleotide polymorphisms associated with cancer or a predisposition to cancer 20 may also be useful for diagnosis or prognosis.

Cancer treatments promote tumor regression by inhibiting tumor cell proliferation, inhibiting angiogenesis (growth of new blood vessels that is necessary to support tumor growth) and/or prohibiting metastasis by reducing tumor cell motility or invasiveness. Therapeutic compositions of the invention may be effective in adult and 25 pediatric oncology including in solid phase tumors/malignancies, locally advanced tumors, human soft tissue sarcomas, metastatic cancer, including lymphatic metastases, blood cell malignancies including multiple myeloma, acute and chronic leukemias, and lymphomas, head and neck cancers including mouth cancer, larynx cancer and thyroid cancer, lung cancers including small cell carcinoma and non-small cell cancers, breast 30 cancers including small cell carcinoma and ductal carcinoma, gastrointestinal cancers

including esophageal cancer, stomach cancer, colon cancer, colorectal cancer and polyps associated with colorectal neoplasia, pancreatic cancers, liver cancer, urologic cancers including bladder cancer and prostate cancer, malignancies of the female genital tract including ovarian carcinoma, uterine (including endometrial) cancers, and solid tumor in the ovarian follicle, kidney cancers including renal cell carcinoma, brain cancers including intrinsic brain tumors, neuroblastoma, astrocytic brain tumors, gliomas, metastatic tumor cell invasion in the central nervous system, bone cancers including osteomas, skin cancers including malignant melanoma, tumor progression of human skin keratinocytes, squamous cell carcinoma, basal cell carcinoma, hemangiopericytoma and Karposi's sarcoma.

Polypeptides, polynucleotides, or modulators of polypeptides of the invention (including inhibitors and stimulators of the biological activity of the polypeptide of the invention) may be administered to treat cancer. Therapeutic compositions can be administered in therapeutically effective dosages alone or in combination with adjuvant cancer therapy such as surgery, chemotherapy, radiotherapy, thermotherapy, and laser therapy, and may provide a beneficial effect, e.g. reducing tumor size, slowing rate of tumor growth, inhibiting metastasis, or otherwise improving overall clinical condition, without necessarily eradicating the cancer.

The composition can also be administered in therapeutically effective amounts as a portion of an anti-cancer cocktail. An anti-cancer cocktail is a mixture of the polypeptide or modulator of the invention with one or more anti-cancer drugs in addition to a pharmaceutically acceptable carrier for delivery. The use of anti-cancer cocktails as a cancer treatment is routine. Anti-cancer drugs that are well known in the art and can be used as a treatment in combination with the polypeptide or modulator of the invention include: Actinomycin D, Aminoglutethimide, Asparaginase, Bleomycin, Busulfan, Carboplatin, Carmustine, Chlorambucil, Cisplatin (cis-DDP), Cyclophosphamide, Cytarabine HCl (Cytosine arabinoside), Dacarbazine, Dactinomycin, Daunorubicin HCl, Doxorubicin HCl, Estramustine phosphate sodium, Etoposide (V16-213), Floxuridine, 5-Fluorouracil (5-Fu), Flutamide, Hydroxyurea (hydroxycarbamide), Ifosfamide, Interferon Alpha-2a, Interferon Alpha-2b, Leuprolide

acetate (LHRH-releasing factor analog), Lomustine, Mechlorethamine HCl (nitrogen mustard), Melphalan, Mercaptopurine, Mesna, Methotrexate (MTX), Mitomycin, Mitoxantrone HCl, Octreotide, Plicamycin, Procarbazine HCl, Streptozocin, Tamoxifen citrate, Thioguanine, Thiotepa, Vinblastine sulfate, Vincristine sulfate,  
5 Amsacrine, Azacitidine, Hexamethylmelamine, Interleukin-2, Mitoguazone, Pentostatin, Semustine, Teniposide, and Vindesine sulfate.

In addition, therapeutic compositions of the invention may be used for prophylactic treatment of cancer. There are hereditary conditions and/or environmental situations (e.g. exposure to carcinogens) known in the art that predispose an individual  
10 to developing cancers. Under these circumstances, it may be beneficial to treat these individuals with therapeutically effective doses of the polypeptide of the invention to reduce the risk of developing cancers.

*In vitro* models can be used to determine the effective doses of the polypeptide of the invention as a potential cancer treatment. These *in vitro* models include  
15 proliferation assays of cultured tumor cells, growth of cultured tumor cells in soft agar (see Freshney, (1987) Culture of Animal Cells: A Manual of Basic Technique, Wiley-Liss, New York, NY Ch 18 and Ch 21), tumor systems in nude mice as described in Giovanella et al., J. Natl. Can. Inst., 52: 921-30 (1974), mobility and invasive potential of tumor cells in Boyden Chamber assays as described in Pilkington et al., Anticancer Res., 17: 4107-9 (1997), and angiogenesis assays such as induction of vascularization of the chick chorioallantoic membrane or induction of vascular endothelial cell migration as described in Ribatta et al., Intl. J. Dev. Biol., 40: 1189-97 (1999) and Li et al., Clin. Exp. Metastasis, 17:423-9 (1999), respectively. Suitable tumor cells lines are available, e.g. from American Type Tissue Culture Collection catalogs.  
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### **5.7.12 RECEPTOR/LIGAND ACTIVITY**

A polypeptide of the present invention may also demonstrate activity as receptor, receptor ligand or inhibitor or agonist of receptor/ligand interactions. A polynucleotide of the invention can encode a polypeptide exhibiting such characteristics.  
30 Examples of such receptors and ligands include, without limitation, cytokine receptors

and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell-cell interactions and their ligands (including without limitation, cellular adhesion molecules (such as selectins, integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses. Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. A protein of the present invention (including, without limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

The activity of a polypeptide of the invention may, among other means, be measured by the following methods:

Suitable assays for receptor-ligand activity include without limitation those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley- Interscience (Chapter 7.28, Measurement of Cellular Adhesion under static conditions 7.28.1- 7.28.22), Takai et al., Proc. Natl. Acad. Sci. USA 84:6864-6868, 1987; Bierer et al., J. Exp. Med. 168:1145-1156, 1988; Rosenstein et al., J. Exp. Med. 169:149-160 1989; Stoltenborg et al., J. Immunol. Methods 175:59-68, 1994; Stitt et al., Cell 80:661-670, 1995.

By way of example, the polypeptides of the invention may be used as a receptor for a ligand(s) thereby transmitting the biological activity of that ligand(s). Ligands may be identified through binding assays, affinity chromatography, dihybrid screening assays, BIACore assays, gel overlay assays, or other methods known in the art.

Studies characterizing drugs or proteins as agonist or antagonist or partial agonists or a partial antagonist require the use of other proteins as competing ligands. The polypeptides of the present invention or ligand(s) thereof may be labeled by being coupled to radioisotopes, colorimetric molecules or a toxin molecules by conventional methods. ("Guide to Protein Purification" Murray P. Deutscher (ed) Methods in Enzymology Vol. 182 (1990) Academic Press, Inc. San Diego). Examples of radioisotopes include, but are not limited to, tritium and carbon-14 . Examples of

colorimetric molecules include, but are not limited to, fluorescent molecules such as fluorescamine, or rhodamine or other colorimetric molecules. Examples of toxins include, but are not limited, to ricin.

5           **5.7.13 DRUG SCREENING**

This invention is particularly useful for screening chemical compounds by using the novel polypeptides or binding fragments thereof in any of a variety of drug screening techniques. The polypeptides or fragments employed in such a test may either be free in solution, affixed to a solid support, borne on a cell surface or located 10 intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the polypeptide or a fragment thereof. Drugs are screened against such transformed cells in competitive binding assays. Such cells, either in viable or fixed form, can be used for standard binding assays. One may measure, for example, the formation of 15 complexes between polypeptides of the invention or fragments and the agent being tested or examine the diminution in complex formation between the novel polypeptides and an appropriate cell line, which are well known in the art.

Sources for test compounds that may be screened for ability to bind to or modulate (i.e., increase or decrease) the activity of polypeptides of the invention 20 include (1) inorganic and organic chemical libraries, (2) natural product libraries, and (3) combinatorial libraries comprised of either random or mimetic peptides, oligonucleotides or organic molecules.

Chemical libraries may be readily synthesized or purchased from a number of commercial sources, and may include structural analogs of known compounds or 25 compounds that are identified as "hits" or "leads" via natural product screening.

The sources of natural product libraries are microorganisms (including bacteria and fungi), animals, plants or other vegetation, or marine organisms, and libraries of mixtures for screening may be created by: (1) fermentation and extraction of broths from soil, plant or marine microorganisms or (2) extraction of the organisms 30 themselves. Natural product libraries include polyketides, non-ribosomal peptides, and

(non-naturally occurring) variants thereof. For a review, see *Science* 282:63-68 (1998).

Combinatorial libraries are composed of large numbers of peptides, oligonucleotides or organic compounds and can be readily prepared by traditional 5 automated synthesis methods, PCR, cloning or proprietary synthetic methods. Of particular interest are peptide and oligonucleotide combinatorial libraries. Still other libraries of interest include peptide, protein, peptidomimetic, multiparallel synthetic collection, recombinatorial, and polypeptide libraries. For a review of combinatorial chemistry and libraries created therefrom, see Myers, *Curr. Opin. Biotechnol.* 8:701-10 707 (1997). For reviews and examples of peptidomimetic libraries, see Al-Obeidi et al., *Mol. Biotechnol.*, 9(3):205-23 (1998); Hruby et al., *Curr Opin Chem Biol*, 1(1):114-19 (1997); Dorner et al., *Bioorg Med Chem*, 4(5):709-15 (1996) (alkylated dipeptides).

Identification of modulators through use of the various libraries described herein 15 permits modification of the candidate "hit" (or "lead") to optimize the capacity of the "hit" to bind a polypeptide of the invention. The molecules identified in the binding assay are then tested for antagonist or agonist activity in *in vivo* tissue culture or animal models that are well known in the art. In brief, the molecules are titrated into a plurality of cell cultures or animals and then tested for either cell/animal death or 20 prolonged survival of the animal/cells.

The binding molecules thus identified may be complexed with toxins, e.g., ricin or cholera, or with other compounds that are toxic to cells such as radioisotopes. The toxin-binding molecule complex is then targeted to a tumor or other cell by the specificity of the binding molecule for a polypeptide of the invention. Alternatively, 25 the binding molecules may be complexed with imaging agents for targeting and imaging purposes.

#### **5.7.14 ASSAY FOR RECEPTOR ACTIVITY**

The invention also provides methods to detect specific binding of a polypeptide 30 e.g. a ligand or a receptor. The art provides numerous assays particularly useful for

identifying previously unknown binding partners for receptor polypeptides of the invention. For example, expression cloning using mammalian or bacterial cells, or dihybrid screening assays can be used to identify polynucleotides encoding binding partners. As another example, affinity chromatography with the appropriate  
5 immobilized polypeptide of the invention can be used to isolate polypeptides that recognize and bind polypeptides of the invention. There are a number of different libraries used for the identification of compounds, and in particular small molecule, that modulate (*i.e.*, increase or decrease) biological activity of a polypeptide of the invention. Ligands for receptor polypeptides of the invention can also be identified by  
10 adding exogenous ligands, or cocktails of ligands to two cells populations that are genetically identical except for the expression of the receptor of the invention: one cell population expresses the receptor of the invention whereas the other does not. The response of the two cell populations to the addition of ligand(s) are then compared. Alternatively, an expression library can be co-expressed with the polypeptide of the invention in cells and assayed for an autocrine response to identify potential ligand(s).  
15 As still another example, BIACore assays, gel overlay assays, or other methods known in the art can be used to identify binding partner polypeptides, including, (1) organic and inorganic chemical libraries, (2) natural product libraries, and (3) combinatorial libraries comprised of random peptides, oligonucleotides or organic molecules.

20 The role of downstream intracellular signaling molecules in the signaling cascade of the polypeptide of the invention can be determined. For example, a chimeric protein in which the cytoplasmic domain of the polypeptide of the invention is fused to the extracellular portion of a protein, whose ligand has been identified, is produced in a host cell. The cell is then incubated with the ligand specific for the extracellular portion  
25 of the chimeric protein, thereby activating the chimeric receptor. Known downstream proteins involved in intracellular signaling can then be assayed for expected modifications i.e. phosphorylation. Other methods known to those in the art can also be used to identify signaling molecules involved in receptor activity.

30 **5.7.15 ANTI-INFLAMMATORY ACTIVITY**

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Compositions of the present invention may also exhibit anti-inflammatory activity. The anti-inflammatory activity may be achieved by providing a stimulus to cells involved in the inflammatory response, by inhibiting or promoting cell-cell interactions (such as, for example, cell adhesion), by inhibiting or promoting 5 chemotaxis of cells involved in the inflammatory process, inhibiting or promoting cell extravasation, or by stimulating or suppressing production of other factors which more directly inhibit or promote an inflammatory response. Compositions with such activities can be used to treat inflammatory conditions including chronic or acute conditions, including without limitation intimation associated with infection (such as septic shock, 10 sepsis or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine-induced lung injury, inflammatory bowel disease, Crohn's disease or resulting from over production of cytokines such as TNF or IL-1. Compositions of the invention may also be useful to treat anaphylaxis and 15 hypersensitivity to an antigenic substance or material. Compositions of this invention may be utilized to prevent or treat conditions such as, but not limited to, sepsis, acute pancreatitis, endotoxin shock, cytokine induced shock, rheumatoid arthritis, chronic inflammatory arthritis, pancreatic cell damage from diabetes mellitus type 1, graft versus host disease, inflammatory bowel disease, inflammation associated with 20 pulmonary disease, other autoimmune disease or inflammatory disease, an antiproliferative agent such as for acute or chronic myelogenous leukemia or in the prevention of premature labor secondary to intrauterine infections.

### **5.7.16 LEUKEMIAS**

Leukemias and related disorders may be treated or prevented by administration 25 of a therapeutic that promotes or inhibits function of the polynucleotides and/or polypeptides of the invention. Such leukemias and related disorders include but are not limited to acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, myeloblastic, promyelocytic, myelomonocytic, monocytic, erythroleukemia, chronic 30 leukemia, chronic myelocytic (granulocytic) leukemia and chronic lymphocytic

leukemia (for a review of such disorders, see Fishman et al., 1985, Medicine, 2d Ed., J.B. Lippincott Co., Philadelphia).

#### **5.7.17 NERVOUS SYSTEM DISORDERS**

5        Nervous system disorders, involving cell types which can be tested for efficacy of intervention with compounds that modulate the activity of the polynucleotides and/or polypeptides of the invention, and which can be treated upon thus observing an indication of therapeutic utility, include but are not limited to nervous system injuries, and diseases or disorders which result in either a disconnection of axons, a diminution  
10      or degeneration of neurons, or demyelination. Nervous system lesions which may be treated in a patient (including human and non-human mammalian patients) according to the invention include but are not limited to the following lesions of either the central (including spinal cord, brain) or peripheral nervous systems:

- 15            (i)      traumatic lesions, including lesions caused by physical injury or associated with surgery, for example, lesions which sever a portion of the nervous system, or compression injuries;
- (ii)     ischemic lesions, in which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia;
- 20            (iii)    infectious lesions, in which a portion of the nervous system is destroyed or injured as a result of infection, for example, by an abscess or associated with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, tuberculosis, syphilis;
- (iv)     degenerative lesions, in which a portion of the nervous system is destroyed or injured as a result of a degenerative process including but not limited to degeneration associated with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic lateral sclerosis;
- 25            (v)      lesions associated with nutritional diseases or disorders, in which a portion of the nervous system is destroyed or injured by a nutritional disorder or disorder of metabolism including but not limited to, vitamin B12 deficiency, folic acid

deficiency, Wernicke disease, tobacco-alcohol amblyopia, Marchiafava-Bignami disease (primary degeneration of the corpus callosum), and alcoholic cerebellar degeneration;

(vi) neurological lesions associated with systemic diseases including but not limited to diabetes (diabetic neuropathy, Bell's palsy), systemic lupus erythematosus, carcinoma, or sarcoidosis;

(vii) lesions caused by toxic substances including alcohol, lead, or particular neurotoxins; and

(viii) demyelinated lesions in which a portion of the nervous system is destroyed or injured by a demyelinating disease including but not limited to multiple sclerosis, human immunodeficiency virus-associated myelopathy, transverse myelopathy or various etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis.

Therapeutics which are useful according to the invention for treatment of a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons. For example, and not by way of limitation, therapeutics which elicit any of the following effects may be useful according to the invention:

(i) increased survival time of neurons in culture;  
(ii) increased sprouting of neurons in culture or *in vivo*;  
20 (iii) increased production of a neuron-associated molecule in culture or *in vivo*, e.g., choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or  
(iv) decreased symptoms of neuron dysfunction *in vivo*.

Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of neurons may be measured by the method set forth in Arakawa et al. (1990, *J. Neurosci.* 10:3507-3515); increased sprouting of neurons may be detected by methods set forth in Pestronk et al. (1980, *Exp. Neurol.* 70:65-82) or Brown et al. (1981, *Ann. Rev. Neurosci.* 4:17-42); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody binding, Northern blot assay, *etc.*, depending on the

molecule to be measured; and motor neuron dysfunction may be measured by assessing the physical manifestation of motor neuron disorder, *e.g.*, weakness, motor neuron conduction velocity, or functional disability.

In specific embodiments, motor neuron disorders that may be treated according  
5 to the invention include but are not limited to disorders such as infarction, infection,  
exposure to toxin, trauma, surgical damage, degenerative disease or malignancy that  
may affect motor neurons as well as other components of the nervous system, as well as  
disorders that selectively affect neurons such as amyotrophic lateral sclerosis, and  
including but not limited to progressive spinal muscular atrophy, progressive bulbar  
10 palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive  
bulbar paralysis of childhood (Fazio-Londe syndrome), poliomyelitis and the post polio  
syndrome, and Hereditary Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

#### **5.7.18 OTHER ACTIVITIES**

A polypeptide of the invention may also exhibit one or more of the following  
15 additional activities or effects: inhibiting the growth, infection or function of, or killing,  
infectious agents, including, without limitation, bacteria, viruses, fungi and other  
parasites; effecting (suppressing or enhancing) bodily characteristics, including, without  
limitation, height, weight, hair color, eye color, skin, fat to lean ratio or other tissue  
20 pigmentation, or organ or body part size or shape (such as, for example, breast  
augmentation or diminution, change in bone form or shape); effecting biorhythms or  
circadian cycles or rhythms; effecting the fertility of male or female subjects; effecting  
the metabolism, catabolism, anabolism, processing, utilization, storage or elimination  
of dietary fat, lipid, protein, carbohydrate, vitamins, minerals, co-factors or other  
25 nutritional factors or component(s); effecting behavioral characteristics, including,  
without limitation, appetite, libido, stress, cognition (including cognitive disorders),  
depression (including depressive disorders) and violent behaviors; providing analgesic  
effects or other pain reducing effects; promoting differentiation and growth of  
embryonic stem cells in lineages other than hematopoietic lineages; hormonal or  
30 endocrine activity; in the case of enzymes, correcting deficiencies of the enzyme and

treating deficiency-related diseases; treatment of hyperproliferative disorders (such as, for example, psoriasis); immunoglobulin-like activity (such as, for example, the ability to bind antigens or complement); and the ability to act as an antigen in a vaccine composition to raise an immune response against such protein or another material or entity which is cross-reactive with such protein.

#### **5.7.19 IDENTIFICATION OF POLYMORPHISMS**

The demonstration of polymorphisms makes possible the identification of such polymorphisms in human subjects and the pharmacogenetic use of this information for diagnosis and treatment. Such polymorphisms may be associated with, e.g., differential predisposition or susceptibility to various disease states (such as disorders involving inflammation or immune response) or a differential response to drug administration, and this genetic information can be used to tailor preventive or therapeutic treatment appropriately. For example, the existence of a polymorphism associated with a predisposition to inflammation or autoimmune disease makes possible the diagnosis of this condition in humans by identifying the presence of the polymorphism.

Polymorphisms can be identified in a variety of ways known in the art which all generally involve obtaining a sample from a patient, analyzing DNA from the sample, optionally involving isolation or amplification of the DNA, and identifying the presence of the polymorphism in the DNA. For example, PCR may be used to amplify an appropriate fragment of genomic DNA which may then be sequenced. Alternatively, the DNA may be subjected to allele-specific oligonucleotide hybridization (in which appropriate oligonucleotides are hybridized to the DNA under conditions permitting detection of a single base mismatch) or to a single nucleotide extension assay (in which an oligonucleotide that hybridizes immediately adjacent to the position of the polymorphism is extended with one or more labeled nucleotides). In addition, traditional restriction fragment length polymorphism analysis (using restriction enzymes that provide differential digestion of the genomic DNA depending on the presence or absence of the polymorphism) may be performed. Arrays with nucleotide sequences of the present invention can be used to detect polymorphisms. The array can comprise

modified nucleotide sequences of the present invention in order to detect the nucleotide sequences of the present invention. In the alternative, any one of the nucleotide sequences of the present invention can be placed on the array to detect changes from those sequences.

5        Alternatively a polymorphism resulting in a change in the amino acid sequence could also be detected by detecting a corresponding change in amino acid sequence of the protein, e.g., by an antibody specific to the variant sequence.

#### **5.7.20 ARTHRITIS AND INFLAMMATION**

10      The immunosuppressive effects of the compositions of the invention against rheumatoid arthritis is determined in an experimental animal model system. The experimental model system is adjuvant induced arthritis in rats, and the protocol is described by J. Holoshitz, et al., 1983, Science, 219:56, or by B. Waksman et al., 1963, Int. Arch. Allergy Appl. Immunol., 23:129. Induction of the disease can be 15 caused by a single injection, generally intradermally, of a suspension of killed Mycobacterium tuberculosis in complete Freund's adjuvant (CFA). The route of injection can vary, but rats may be injected at the base of the tail with an adjuvant mixture. The polypeptide is administered in phosphate buffered solution (PBS) at a dose of about 1-5 mg/kg. The control consists of administering PBS only.

20      The procedure for testing the effects of the test compound would consist of intradermally injecting killed Mycobacterium tuberculosis in CFA followed by immediately administering the test compound and subsequent treatment every other day until day 24. At 14, 15, 18, 20, 22, and 24 days after injection of Mycobacterium CFA, an overall arthritis score may be obtained as described by J. Holoskitz above. An 25 analysis of the data would reveal that the test compound would have a dramatic affect on the swelling of the joints as measured by a decrease of the arthritis score.

#### **5.8 THERAPEUTIC METHODS**

The compositions (including polypeptide fragments, analogs, variants and antibodies or other binding partners or modulators including antisense polynucleotides) of the invention have numerous applications in a variety of therapeutic methods.

Examples of therapeutic applications include, but are not limited to, those exemplified

5 herein.

### **5.8.1 EXAMPLE**

One embodiment of the invention is the administration of an effective amount of the LRR protein-like polypeptides or other composition of the invention to individuals

10 affected by a disease or disorder that can be modulated by regulating the peptides of the invention. While the mode of administration is not particularly important, parenteral administration is preferred. An exemplary mode of administration is to deliver an

intravenous bolus. The dosage of LRR protein-like polypeptides or other composition of the invention will normally be determined by the prescribing physician. It is to be

15 expected that the dosage will vary according to the age, weight, condition and response of the individual patient. Typically, the amount of polypeptide administered per dose will be in the range of about 0.01 $\mu$ g/kg to 100 mg/kg of body weight, with the preferred dose being about 0.1 $\mu$ g/kg to 10 mg/kg of patient body weight. For

20 parenteral administration, LRR protein-like polypeptides of the invention will be formulated in an injectable form combined with a pharmaceutically acceptable parenteral vehicle. Such vehicles are well known in the art and examples include

water, saline, Ringer's solution, dextrose solution, and solutions consisting of small amounts of the human serum albumin. The vehicle may contain minor amounts of additives that maintain the isotonicity and stability of the polypeptide or other active

25 ingredient. The preparation of such solutions is within the skill of the art.

## **5.9 PHARMACEUTICAL FORMULATIONS AND ROUTES OF ADMINISTRATION**

A protein or other composition of the present invention (from whatever source derived, including without limitation from recombinant and non-recombinant sources and including antibodies and other binding partners of the polypeptides of the invention) may be administered to a patient in need, by itself, or in pharmaceutical compositions where it is mixed with suitable carriers or excipient(s) at doses to treat or ameliorate a variety of disorders. Such a composition may optionally contain (in addition to protein or other active ingredient and a carrier) diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration. The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, TNF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IFN, TNF0, TNF1, TNF2, G-CSF, Meg-CSF, thrombopoietin, stem cell factor, and erythropoietin. In further compositions, proteins of the invention may be combined with other agents beneficial to the treatment of the disease or disorder in question. These agents include various growth factors such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), transforming growth factors (TGF- $\alpha$  and TGF- $\beta$ ), insulin-like growth factor (IGF), as well as cytokines described herein.

The pharmaceutical composition may further contain other agents which either enhance the activity of the protein or other active ingredient or complement its activity or use in treatment. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with protein or other active ingredient of the invention, or to minimize side effects. Conversely, protein or other active ingredient of the present invention may be included in formulations of the particular clotting factor, cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti- inflammatory agent to minimize side effects of the clotting factor, cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent (such as IL-1Ra, IL-

1 Hy1, IL-1 Hy2, anti-TNF, corticosteroids, immunosuppressive agents). A protein of the present invention may be active in multimers (e.g., heterodimers or homodimers) or complexes with itself or other proteins. As a result, pharmaceutical compositions of the invention may comprise a protein of the invention in such multimeric or complexed

5 form.

As an alternative to being included in a pharmaceutical composition of the invention including a first protein, a second protein or a therapeutic agent may be concurrently administered with the first protein (e.g., at the same time, or at differing times provided that therapeutic concentrations of the combination of agents is achieved

10 at the treatment site). Techniques for formulation and administration of the compounds of the instant application may be found in "Remington's Pharmaceutical Sciences,"

Mack Publishing Co., Easton, PA, latest edition. A therapeutically effective dose further refers to that amount of the compound sufficient to result in amelioration of symptoms, *e.g.*, treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to an individual active ingredient, administered alone, a therapeutically effective dose refers to that ingredient alone. When applied to a combination, a therapeutically effective dose refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of protein or other active ingredient of the present invention is administered to a mammal having a condition to be treated. Protein or other active ingredient of the present invention may be administered in accordance with the method of the invention either alone or in combination with other therapies such as treatments employing cytokines, lymphokines or other hematopoietic factors. When co- administered with one or more cytokines, lymphokines or other hematopoietic factors, protein or other active ingredient of the present invention may be administered either simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered

sequentially, the attending physician will decide on the appropriate sequence of administering protein or other active ingredient of the present invention in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

5

### **5.9.1 ROUTES OF ADMINISTRATION**

Suitable routes of administration may, for example, include oral, rectal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections. Administration of protein or other active ingredient of the present invention used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, topical application or cutaneous, subcutaneous, intraperitoneal, parenteral or intravenous injection.

10 15 Intravenous administration to the patient is preferred.

Alternately, one may administer the compound in a local rather than systemic manner, for example, via injection of the compound directly into a arthritic joints or in fibrotic tissue, often in a depot or sustained release formulation. In order to prevent the scarring process frequently occurring as complication of glaucoma surgery, the 20 compounds may be administered topically, for example, as eye drops. Furthermore, one may administer the drug in a targeted drug delivery system, for example, in a liposome coated with a specific antibody, targeting, for example, arthritic or fibrotic tissue. The liposomes will be targeted to and taken up selectively by the afflicted tissue.

25 The polypeptides of the invention are administered by any route that delivers an effective dosage to the desired site of action. The determination of a suitable route of administration and an effective dosage for a particular indication is within the level of skill in the art. Preferably for wound treatment, one administers the therapeutic compound directly to the site. Suitable dosage ranges for the polypeptides of the 30 invention can be extrapolated from these dosages or from similar studies in appropriate

animal models. Dosages can then be adjusted as necessary by the clinician to provide maximal therapeutic benefit.

### **5.9.2 COMPOSITIONS/FORMULATIONS**

5 Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. These pharmaceutical compositions may be manufactured in a manner that is itself known,  
10 e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes. Proper formulation is dependent upon the route of administration chosen. When a therapeutically effective amount of protein or other active ingredient of the present invention is administered orally, protein or other active ingredient of the present  
15 invention will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% protein or other active ingredient of the present invention, and preferably from about 25 to 90% protein or other active  
20 ingredient of the present invention. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or  
25 polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of protein or other active ingredient of the present invention, and preferably from about 1 to 50% protein or other active ingredient of the present invention.

When a therapeutically effective amount of protein or other active ingredient of  
30 the present invention is administered by intravenous, cutaneous or subcutaneous

injection, protein or other active ingredient of the present invention will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein or other active ingredient solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred 5 pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to protein or other active ingredient of the present invention, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present 10 invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art. For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are 15 used in the formulation. Such penetrants are generally known in the art.

For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral 20 ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, 25 maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Dragee cores are provided with suitable coatings. For this purpose, 30 concentrated sugar solutions may be used, which may optionally contain gum arabic,

talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

5 Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active

10 compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration. For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

15 For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a

20 pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, *e.g.*, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch. The compounds may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion.

25 Formulations for injection may be presented in unit dosage form, *e.g.*, in ampules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous

30 solutions of the active compounds in water-soluble form. Additionally, suspensions of

the active compounds may be prepared as appropriate oily injection suspensions.

Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides. In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

A pharmaceutical carrier for the hydrophobic compounds of the invention is a co-solvent system comprising benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer, and an aqueous phase. The co-solvent system may be the VPD co-solvent system. VPD is a solution of 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant polysorbate 80, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. The VPD co-solvent system (VPD:5W) consists of VPD diluted 1:1 with a 5% dextrose in water solution. This co-solvent system dissolves hydrophobic compounds well, and itself produces low toxicity upon systemic administration. Naturally, the proportions of a co-solvent system may be varied considerably without destroying its solubility and toxicity characteristics. Furthermore, the identity of the co-solvent components may be varied: for example, other low-toxicity nonpolar surfactants may be used instead of polysorbate 80; the fraction size of polyethylene

glycol may be varied; other biocompatible polymers may replace polyethylene glycol, e.g. polyvinyl pyrrolidone; and other sugars or polysaccharides may substitute for dextrose. Alternatively, other delivery systems for hydrophobic pharmaceutical compounds may be employed. Liposomes and emulsions are well known examples of 5 delivery vehicles or carriers for hydrophobic drugs. Certain organic solvents such as dimethylsulfoxide also may be employed, although usually at the cost of greater toxicity. Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various types of sustained-release materials have been established 10 and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein or other active ingredient stabilization may be employed.

15 The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols. Many of the active ingredients of the invention may be provided as salts with pharmaceutically compatible 20 counter ions. Such pharmaceutically acceptable base addition salts are those salts which retain the biological effectiveness and properties of the free acids and which are obtained by reaction with inorganic or organic bases such as sodium hydroxide, magnesium hydroxide, ammonia, trialkylamine, dialkylamine, monoalkylamine, dibasic amino acids, sodium acetate, potassium benzoate, triethanol amine and the like.

25 The pharmaceutical composition of the invention may be in the form of a complex of the protein(s) or other active ingredient of present invention along with protein or peptide antigens. The protein and/or peptide antigen will deliver a stimulatory signal to both B and T lymphocytes. B lymphocytes will respond to antigen through their surface immunoglobulin receptor. T lymphocytes will respond to antigen 30 through the T cell receptor (TCR) following presentation of the antigen by MHC

proteins. MHC and structurally related proteins including those encoded by class I and class II MHC genes on host cells will serve to present the peptide antigen(s) to T lymphocytes. The antigen components could also be supplied as purified MHC-peptide complexes alone or with co-stimulatory molecules that can directly signal T cells.

5 Alternatively antibodies able to bind surface immunoglobulin and other molecules on B cells as well as antibodies able to bind the TCR and other molecules on T cells can be combined with the pharmaceutical composition of the invention.

The pharmaceutical composition of the invention may be in the form of a liposome in which protein of the present invention is combined, in addition to other 10 pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithins, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within 15 the level of skill in the art, as disclosed, for example, in U.S. Patent Nos. 4,235,871; 4,501,728; 4,837,028; and 4,737,323, all of which are incorporated herein by reference.

The amount of protein or other active ingredient of the present invention in the pharmaceutical composition of the present invention will depend upon the nature and 20 severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of protein or other active ingredient of the present invention with which to treat each individual patient. Initially, the attending physician will administer low doses of protein or other active ingredient of the present invention and observe the patient's 25 response. Larger doses of protein or other active ingredient of the present invention may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.01  $\mu$ g to about 100 mg (preferably about 0.1  $\mu$ g to about 10 mg, 30 more preferably about 0.1  $\mu$ g to about 1 mg) of protein or other active ingredient of the

present invention per kg body weight. For compositions of the present invention which are useful for bone, cartilage, tendon or ligament regeneration, the therapeutic method includes administering the composition topically, systematically, or locally as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone, cartilage or tissue damage. Topical administration may be suitable for wound healing and tissue repair. Therapeutically useful agents other than a protein or other active ingredient of the invention which may also optionally be included in the composition as described above, may alternatively or additionally, be administered simultaneously or sequentially with the composition in the methods of the invention. Preferably for bone and/or cartilage formation, the composition would include a matrix capable of delivering the protein-containing or other active ingredient-containing composition to the site of bone and/or cartilage damage, providing a structure for the developing bone and cartilage and optimally capable of being resorbed into the body. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular application of the compositions will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalcium phosphate, hydroxyapatite, polylactic acid, polyglycolic acid and polyanhydrides. Other potential materials are biodegradable and biologically well-defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalcium phosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size,

particle shape, and biodegradability. Presently preferred is a 50:50 (mole weight) copolymer of lactic acid and glycolic acid in the form of porous particles having diameters ranging from 150 to 800 microns. In some applications, it will be useful to utilize a sequestering agent, such as carboxymethyl cellulose or autologous blood clot,

5 to prevent the protein compositions from disassociating from the matrix.

A preferred family of sequestering agents is cellulosic materials such as alkylcelluloses (including hydroxyalkylcelluloses), including methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropyl-methylcellulose, and carboxymethylcellulose, the most preferred being cationic salts of

10 carboxymethylcellulose (CMC). Other preferred sequestering agents include hyaluronic acid, sodium alginate, poly(ethylene glycol), polyoxyethylene oxide, carboxyvinyl polymer and poly(vinyl alcohol). The amount of sequestering agent useful herein is 0.5-20 wt %, preferably 1-10 wt % based on total formulation weight, which represents the amount necessary to prevent desorption of the protein from the

15 polymer matrix and to provide appropriate handling of the composition, yet not so much that the progenitor cells are prevented from infiltrating the matrix, thereby providing the protein the opportunity to assist the osteogenic activity of the progenitor cells. In further compositions, proteins or other active ingredient of the invention may be combined with other agents beneficial to the treatment of the bone and/or cartilage

20 defect, wound, or tissue in question. These agents include various growth factors such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), transforming growth factors (TGF- $\alpha$  and TGF- $\beta$ ), and insulin-like growth factor (IGF).

The therapeutic compositions are also presently valuable for veterinary applications. Particularly domestic animals and thoroughbred horses, in addition to

25 humans, are desired patients for such treatment with proteins or other active ingredient of the present invention. The dosage regimen of a protein-containing pharmaceutical composition to be used in tissue regeneration will be determined by the attending physician considering various factors which modify the action of the proteins, *e.g.*, amount of tissue weight desired to be formed, the site of damage, the condition of the

30 damaged tissue, the size of a wound, type of damaged tissue (*e.g.*, bone), the patient's

age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and with inclusion of other proteins in the pharmaceutical composition. For example, the addition of other known growth factors, such as IGF I (insulin like growth factor I), to the final composition, may also effect the dosage. Progress can be monitored by periodic assessment of tissue/bone growth and/or repair, for example, X-rays, histomorphometric determinations and tetracycline labeling.

Polynucleotides of the present invention can also be used for gene therapy. Such polynucleotides can be introduced either *in vivo* or *ex vivo* into cells for expression in a mammalian subject. Polynucleotides of the invention may also be administered by other known methods for introduction of nucleic acid into a cell or organism (including, without limitation, in the form of viral vectors or naked DNA). Cells may also be cultured *ex vivo* in the presence of proteins of the present invention in order to proliferate or to produce a desired effect on or activity in such cells. Treated cells can then be introduced *in vivo* for therapeutic purposes.

### **5.9.3 EFFECTIVE DOSAGE**

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. More specifically, a therapeutically effective amount means an amount effective to prevent development of or to alleviate the existing symptoms of the subject being treated. Determination of the effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from appropriate *in vitro* assays. For example, a dose can be formulated in animal models to achieve a circulating concentration range that can be used to more accurately determine useful doses in humans. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes the IC<sub>50</sub> as determined in cell culture (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of the

protein's biological activity). Such information can be used to more accurately determine useful doses in humans.

A therapeutically effective dose refers to that amount of the compound that results in amelioration of symptoms or a prolongation of survival in a patient. Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD<sub>50</sub> (the dose lethal to 50% of the population) and the ED<sub>50</sub> (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio between LD<sub>50</sub> and ED<sub>50</sub>. Compounds which exhibit high therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED<sub>50</sub> with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. See, *e.g.*, Fingl et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1. Dosage amount and interval may be adjusted individually to provide plasma levels of the active moiety which are sufficient to maintain the desired effects, or minimal effective concentration (MEC). The MEC will vary for each compound but can be estimated from *in vitro* data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. However, HPLC assays or bioassays can be used to determine plasma concentrations.

Dosage intervals can also be determined using MEC value. Compounds should be administered using a regimen which maintains plasma levels above the MEC for 10-90% of the time, preferably between 30-90% and most preferably between 50-90%. In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration.

An exemplary dosage regimen for polypeptides or other compositions of the invention will be in the range of about 0.01 µg/kg to 100 mg/kg of body weight daily,

with the preferred dose being about 0.1 µg/kg to 25 mg/kg of patient body weight daily, varying in adults and children. Dosing may be once daily, or equivalent doses may be delivered at longer or shorter intervals.

The amount of composition administered will, of course, be dependent on the  
5 subject being treated, on the subject's age and weight, the severity of the affliction, the manner of administration and the judgment of the prescribing physician.

#### **5.9.4 PACKAGING**

The compositions may, if desired, be presented in a pack or dispenser device  
10 which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. Compositions comprising a compound of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and  
15 labeled for treatment of an indicated condition.

#### **5.10 ANTIBODIES**

Another aspect of the invention is an antibody that specifically binds the polypeptide of the invention. Such antibodies include monoclonal and polyclonal  
20 antibodies, single chain antibodies, chimeric antibodies, bifunctional/bispecific antibodies, humanized antibodies, human antibodies, and complementary determining region (CDR)-grafted antibodies, including compounds which include CDR and/or antigen-binding sequences, which specifically recognize a polypeptide of the invention. Preferred antibodies of the invention are human antibodies which are produced and  
25 identified according to methods described in WO93/11236, published June 20, 1993, which is incorporated herein by reference in its entirety. Antibody fragments, including Fab, Fab', F(ab')<sub>2</sub>, and F<sub>v</sub>, are also provided by the invention. The term "specific for" indicates that the variable regions of the antibodies of the invention recognize and bind polypeptides of the invention exclusively (*i.e.*, able to distinguish  
30 the polypeptide of the invention from other similar polypeptides despite sequence

identity, homology, or similarity found in the family of polypeptides), but may also interact with other proteins (for example, *S. aureus* protein A or other antibodies in ELISA techniques) through interactions with sequences outside the variable region of the antibodies, and in particular, in the constant region of the molecule. Screening assays to determine binding specificity of an antibody of the invention are well known and routinely practiced in the art. For a comprehensive discussion of such assays, see Harlow et al. (Eds), Antibodies A Laboratory Manual; Cold Spring Harbor Laboratory; Cold Spring Harbor , NY (1988), Chapter 6. Antibodies that recognize and bind fragments of the polypeptides of the invention are also contemplated, provided that the antibodies are first and foremost specific for, as defined above, full length polypeptides of the invention. As with antibodies that are specific for full length polypeptides of the invention, antibodies of the invention that recognize fragments are those which can distinguish polypeptides from the same family of polypeptides despite inherent sequence identity, homology, or similarity found in the family of proteins. Antibodies of the invention can be produced using any method well known and routinely practiced in the art.

Non-human antibodies may be humanized by any methods known in the art. In one method, the non-human CDRs are inserted into a human antibody or consensus antibody framework sequence. Further changes can then be introduced into the antibody framework to modulate affinity or immunogenicity.

Antibodies of the invention are useful for, for example, therapeutic purposes (by modulating activity of a polypeptide of the invention), diagnostic purposes to detect or quantitate a polypeptide of the invention, as well as purification of a polypeptide of the invention. Kits comprising an antibody of the invention for any of the purposes described herein are also comprehended. In general, a kit of the invention also includes a control antigen for which the antibody is immunospecific. The invention further provides a hybridoma that produces an antibody according to the invention. Antibodies of the invention are useful for detection and/or purification of the polypeptides of the invention.

Polypeptides of the invention may also be used to immunize animals to obtain polyclonal and monoclonal antibodies which specifically react with the protein. Such antibodies may be obtained using either the entire protein or fragments thereof as an immunogen. The peptide immunogens additionally may contain a cysteine residue at 5 the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Methods for synthesizing such peptides are known in the art, for example, as in R. P. Merrifield, J. Amer. Chem. Soc. 85, 2149-2154 (1963); J. L. Krstenansky, et al., FEBS Lett. 211, 10 (1987).

Monoclonal antibodies binding to the protein of the invention may be useful 10 diagnostic agents for the immunodetection of the protein. Neutralizing monoclonal antibodies binding to the protein may also be useful therapeutics for both conditions associated with the protein and also in the treatment of some forms of cancer where abnormal expression of the protein is involved. In the case of cancerous cells or 15 leukemic cells, neutralizing monoclonal antibodies against the protein may be useful in detecting and preventing the metastatic spread of the cancerous cells, which may be mediated by the protein. In general, techniques for preparing polyclonal and monoclonal antibodies as well as hybridomas capable of producing the desired antibody are well known in the art (Campbell, A.M., Monoclonal Antibodies Technology: 20 Laboratory Techniques in Biochemistry and Molecular Biology, Elsevier Science Publishers, Amsterdam, The Netherlands (1984); St. Groth et al., J. Immunol. 35:1-21 (1990); Kohler and Milstein, Nature 256:495-497 (1975)), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., Immunology Today 4:72 (1983); Cole et al., in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc. (1985), pp. 77-96).

25 Any animal (mouse, rabbit, etc.) which is known to produce antibodies can be immunized with a peptide or polypeptide of the invention. Methods for immunization are well known in the art. Such methods include subcutaneous or intraperitoneal injection of the polypeptide. One skilled in the art will recognize that the amount of the protein encoded by the ORF of the present invention used for immunization will vary 30 based on the animal which is immunized, the antigenicity of the peptide and the site of

injection. The protein that is used as an immunogen may be modified or administered in an adjuvant in order to increase the protein's antigenicity. Methods of increasing the antigenicity of a protein are well known in the art and include, but are not limited to, coupling the antigen with a heterologous protein (such as globulin or  $\beta$ -galactosidase) or 5 through the inclusion of an adjuvant during immunization.

For monoclonal antibodies, spleen cells from the immunized animals are removed, fused with myeloma cells, such as SP2/0-Ag14 myeloma cells, and allowed to become monoclonal antibody producing hybridoma cells. Any one of a number of methods well known in the art can be used to identify the hybridoma cell which 10 produces an antibody with the desired characteristics. These include screening the hybridomas with an ELISA assay, Western blot analysis, or radioimmunoassay (Lutz et al., Exp. Cell Research. 175:109-124 (1988)). Hybridomas secreting the desired antibodies are cloned and the class and subclass is determined using procedures known in the art (Campbell, A.M., Monoclonal Antibody Technology: Laboratory 15 Techniques in Biochemistry and Molecular Biology, Elsevier Science Publishers, Amsterdam, The Netherlands (1984)). Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to proteins of the present invention.

For polyclonal antibodies, antibody-containing antiserum is isolated from the 20 immunized animal and is screened for the presence of antibodies with the desired specificity using one of the above-described procedures. The present invention further provides the above- described antibodies in detectably labeled form. Antibodies can be detectably labeled through the use of radioisotopes, affinity labels (such as biotin, avidin, etc.), enzymatic labels (such as horseradish peroxidase, alkaline phosphatase, 25 etc.) fluorescent labels (such as FITC or rhodamine, etc.), paramagnetic atoms, etc. Procedures for accomplishing such labeling are well known in the art; for example, see (Sternberger, L.A. et al., J. Histochem. Cytochem. 18:315 (1970); Bayer, E.A. et al., Meth. Enzym. 62:308 (1979); Engval, E. et al., Immunol. 109:129 (1972); Goding, J.W. J. Immunol. Meth. 13:215 (1976)).

The labeled antibodies of the present invention can be used for *in vitro*, *in vivo*, and *in situ* assays to identify cells or tissues in which a fragment of the polypeptide of interest is expressed. The antibodies may also be used directly in therapies or other diagnostics. The present invention further provides the above-described antibodies  
5 immobilized on a solid support. Examples of such solid supports include plastics such as polycarbonate, complex carbohydrates such as agarose and Sepharose®, acrylic resins and such as polyacrylamide and latex beads. Techniques for coupling antibodies to such solid supports are well known in the art (Weir, D.M. et al., "Handbook of Experimental Immunology" 4th Ed., Blackwell Scientific Publications, Oxford,  
10 England, Chapter 10 (1986); Jacoby, W.D. et al., Meth. Enzym. 34 Academic Press, N.Y. (1974)). The immobilized antibodies of the present invention can be used for *in vitro*, *in vivo*, and *in situ* assays as well as for immuno-affinity purification of the proteins of the present invention.

15 **5.11 COMPUTER READABLE SEQUENCES**

In one application of this embodiment, a nucleotide sequence of the present invention can be recorded on computer readable media. As used herein, "computer readable media" refers to any medium which can be read and accessed directly by a computer. Such media include, but are not limited to: magnetic storage media, such as  
20 floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media. A skilled artisan can readily appreciate how any of the presently known computer readable mediums can be used to create a manufacture comprising computer readable medium having recorded thereon a nucleotide sequence of the present invention. As used herein, "recorded" refers to a  
25 process for storing information on computer readable medium. A skilled artisan can readily adopt any of the presently known methods for recording information on computer readable medium to generate manufactures comprising the nucleotide sequence information of the present invention.

A variety of data storage structures are available to a skilled artisan for creating a computer readable medium having recorded thereon a nucleotide sequence of the present invention. The choice of the data storage structure will generally be based on the means chosen to access the stored information. In addition, a variety of data processor programs and formats can be used to store the nucleotide sequence information of the present invention on computer readable medium. The sequence information can be represented in a word processing text file, formatted in commercially-available software such as WordPerfect and Microsoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like. A skilled artisan can readily adapt any number of data processor structuring formats (*e.g.* text file or database) in order to obtain computer readable medium having recorded thereon the nucleotide sequence information of the present invention.

By providing any of the nucleotide sequences SEQ ID NO: 1-3 or 5 or a representative fragment thereof; or a nucleotide sequence at least 95% identical to any of the nucleotide sequences of the SEQ ID NO: 1-3 or 5 in computer readable form, a skilled artisan can routinely access the sequence information for a variety of purposes. Computer software is publicly available which allows a skilled artisan to access sequence information provided in a computer readable medium. The examples which follow demonstrate how software which implements the BLAST (Altschul et al., J. Mol. Biol. 215:403-410 (1990)) and BLAZE (Brutlag et al., Comp. Chem. 17:203-207 (1993)) search algorithms on a Sybase system is used to identify open reading frames (ORFs) within a nucleic acid sequence. Such ORFs may be protein encoding fragments and may be useful in producing commercially important proteins such as enzymes used in fermentation reactions and in the production of commercially useful metabolites.

As used herein, "a computer-based system" refers to the hardware means, software means, and data storage means used to analyze the nucleotide sequence information of the present invention. The minimum hardware means of the computer-based systems of the present invention comprises a central processing unit (CPU), input means, output means, and data storage means. A skilled artisan can readily appreciate

that any one of the currently available computer-based systems are suitable for use in the present invention. As stated above, the computer-based systems of the present invention comprise a data storage means having stored therein a nucleotide sequence of the present invention and the necessary hardware means and software means for supporting and implementing a search means. As used herein, "data storage means" refers to memory which can store nucleotide sequence information of the present invention, or a memory access means which can access manufactures having recorded thereon the nucleotide sequence information of the present invention.

As used herein, "search means" refers to one or more programs which are implemented on the computer-based system to compare a target sequence or target structural motif with the sequence information stored within the data storage means. Search means are used to identify fragments or regions of a known sequence which match a particular target sequence or target motif. A variety of known algorithms are disclosed publicly and a variety of commercially available software for conducting search means are and can be used in the computer-based systems of the present invention. Examples of such software includes, but is not limited to, Smith-Waterman, MacPattern (EMBL), BLASTN and BLASTA (NPOLYPEPTIDEIA). A skilled artisan can readily recognize that any one of the available algorithms or implementing software packages for conducting homology searches can be adapted for use in the present computer-based systems. As used herein, a "target sequence" can be any nucleic acid or amino acid sequence of six or more nucleotides or two or more amino acids. A skilled artisan can readily recognize that the longer a target sequence is, the less likely a target sequence will be present as a random occurrence in the database. The most preferred sequence length of a target sequence is from about 10 to 100 amino acids, or from about 30 to 300 nucleotide residues. However, it is well recognized that searches for commercially important fragments, such as sequence fragments involved in gene expression and protein processing, may be of shorter length.

As used herein, "a target structural motif," or "target motif," refers to any rationally selected sequence or combination of sequences in which the sequence(s) are chosen based on a three-dimensional configuration which is formed upon the folding of

the target motif. There are a variety of target motifs known in the art. Protein target motifs include, but are not limited to, enzyme active sites and signal sequences.

Nucleic acid target motifs include, but are not limited to, promoter sequences, hairpin structures and inducible expression elements (protein binding sequences).

5

### **5.12 TRIPLE HELIX FORMATION**

In addition, the fragments of the present invention, as broadly described, can be used to control gene expression through triple helix formation or antisense DNA or RNA, both of which methods are based on the binding of a polynucleotide sequence to 10 DNA or RNA. Polynucleotides suitable for use in these methods are usually 20 to 40 bases in length and are designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 15241:456 (1988); and Dervan et al., Science 251:1360 (1991)) or to the mRNA itself (antisense - Olmno, J. Neurochem. 56:560 (1991); Oligodeoxynucleotides 15 as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)).

Triple helix-formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques have been demonstrated to be effective in model systems. Information contained in the sequences of the present invention is necessary for the 20 design of an antisense or triple helix oligonucleotide.

### **5.13 DIAGNOSTIC ASSAYS AND KITS**

The present invention further provides methods to identify the presence or expression of one of the ORFs of the present invention, or homolog thereof, in a test 25 sample, using a nucleic acid probe or antibodies of the present invention, optionally conjugated or otherwise associated with a suitable label.

In general, methods for detecting a polynucleotide of the invention can comprise contacting a sample with a compound that binds to and forms a complex with the polynucleotide for a period sufficient to form the complex, and detecting the complex, 30 so that if a complex is detected, a polynucleotide of the invention is detected in the

sample. Such methods can also comprise contacting a sample under stringent hybridization conditions with nucleic acid primers that anneal to a polynucleotide of the invention under such conditions, and amplifying annealed polynucleotides, so that if a polynucleotide is amplified, a polynucleotide of the invention is detected in the sample.

5 In general, methods for detecting a polypeptide of the invention can comprise contacting a sample with a compound that binds to and forms a complex with the polypeptide for a period sufficient to form the complex, and detecting the complex, so that if a complex is detected, a polypeptide of the invention is detected in the sample.

In detail, such methods comprise incubating a test sample with one or more of 10 the antibodies or one or more of the nucleic acid probes of the present invention and assaying for binding of the nucleic acid probes or antibodies to components within the test sample.

Conditions for incubating a nucleic acid probe or antibody with a test sample vary. Incubation conditions depend on the format employed in the assay, the detection 15 methods employed, and the type and nature of the nucleic acid probe or antibody used in the assay. One skilled in the art will recognize that any one of the commonly available hybridization, amplification or immunological assay formats can readily be adapted to employ the nucleic acid probes or antibodies of the present invention.

Examples of such assays can be found in Chard, T., An Introduction to 20 Radioimmunoassay and Related Techniques, Elsevier Science Publishers, Amsterdam, The Netherlands (1986); Bullock, G.R. et al., Techniques in Immunocytochemistry, Academic Press, Orlando, FL Vol. 1 (1982), Vol. 2 (1983), Vol. 3 (1985); Tijssen, P., Practice and Theory of immunoassays: Laboratory Techniques in Biochemistry and Molecular Biology, Elsevier Science Publishers, Amsterdam, The Netherlands (1985).

25 The test samples of the present invention include cells, protein or membrane extracts of cells, or biological fluids such as sputum, blood, serum, plasma, or urine. The test sample used in the above-described method will vary based on the assay format, nature of the detection method and the tissues, cells or extracts used as the sample to be assayed. Methods for preparing protein extracts or membrane extracts of cells are well

known in the art and can be readily be adapted in order to obtain a sample which is compatible with the system utilized.

In another embodiment of the present invention, kits are provided which contain the necessary reagents to carry out the assays of the present invention. Specifically, 5 the invention provides a compartment kit to receive, in close confinement, one or more containers which comprises: (a) a first container comprising one of the probes or antibodies of the present invention; and (b) one or more other containers comprising one or more of the following: wash reagents, reagents capable of detecting presence of a bound probe or antibody.

10 In detail, a compartment kit includes any kit in which reagents are contained in separate containers. Such containers include small glass containers, plastic containers or strips of plastic or paper. Such containers allows one to efficiently transfer reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated, and the agents or solutions of each container can be added in a 15 quantitative fashion from one compartment to another. Such containers will include a container which will accept the test sample, a container which contains the antibodies used in the assay, containers which contain wash reagents (such as phosphate buffered saline, Tris-buffers, etc.), and containers which contain the reagents used to detect the bound antibody or probe. Types of detection reagents include labeled nucleic acid 20 probes, labeled secondary antibodies, or in the alternative, if the primary antibody is labeled, the enzymatic, or antibody binding reagents which are capable of reacting with the labeled antibody. One skilled in the art will readily recognize that the disclosed probes and antibodies of the present invention can be readily incorporated into one of the established kit formats which are well known in the art.

25

#### **5.14 MEDICAL IMAGING**

The novel polypeptides and binding partners of the invention are useful in medical imaging of sites expressing the molecules of the invention (e.g., where the polypeptide of the invention is involved in the immune response, for imaging sites of 30 inflammation or infection). See, e.g., Kunkel et al., U.S. Pat. NO. 5,413,778. Such

methods involve chemical attachment of a labeling or imaging agent, administration of the labeled polypeptide to a subject in a pharmaceutically acceptable carrier, and imaging the labeled polypeptide *in vivo* at the target site.

## 5 5.15 SCREENING ASSAYS

Using the isolated proteins and polynucleotides of the invention, the present invention further provides methods of obtaining and identifying agents which bind to a polypeptide encoded by an ORF corresponding to any of the nucleotide sequences set forth in the SEQ ID NO: 1-3 or 5, or bind to a specific domain of the polypeptide encoded by the nucleic acid. In detail, said method comprises the steps of:

- (a) contacting an agent with an isolated protein encoded by an ORF of the present invention, or nucleic acid of the invention; and
- (b) determining whether the agent binds to said protein or said nucleic acid.

In general, therefore, such methods for identifying compounds that bind to a polynucleotide of the invention can comprise contacting a compound with a polynucleotide of the invention for a time sufficient to form a polynucleotide/compound complex, and detecting the complex, so that if a polynucleotide/compound complex is detected, a compound that binds to a polynucleotide of the invention is identified.

Likewise, in general, therefore, such methods for identifying compounds that bind to a polypeptide of the invention can comprise contacting a compound with a polypeptide of the invention for a time sufficient to form a polypeptide/compound complex, and detecting the complex, so that if a polypeptide/compound complex is detected, a compound that binds to a polynucleotide of the invention is identified.

Methods for identifying compounds that bind to a polypeptide of the invention can also comprise contacting a compound with a polypeptide of the invention in a cell for a time sufficient to form a polypeptide/compound complex, wherein the complex drives expression of a receptor gene sequence in the cell, and detecting the complex by detecting reporter gene sequence expression, so that if a polypeptide/compound complex is detected, a compound that binds a polypeptide of the invention is identified.

Compounds identified via such methods can include compounds which modulate the activity of a polypeptide of the invention (that is, increase or decrease its activity, relative to activity observed in the absence of the compound). Alternatively, compounds identified via such methods can include compounds which modulate the expression of a polynucleotide of the invention (that is, increase or decrease expression relative to expression levels observed in the absence of the compound). Compounds, such as compounds identified via the methods of the invention, can be tested using standard assays well known to those of skill in the art for their ability to modulate activity/expression.

The agents screened in the above assay can be, but are not limited to, peptides, carbohydrates, vitamin derivatives, or other pharmaceutical agents. The agents can be selected and screened at random or rationally selected or designed using protein modeling techniques.

For random screening, agents such as peptides, carbohydrates, pharmaceutical agents and the like are selected at random and are assayed for their ability to bind to the protein encoded by the ORF of the present invention. Alternatively, agents may be rationally selected or designed. As used herein, an agent is said to be "rationally selected or designed" when the agent is chosen based on the configuration of the particular protein. For example, one skilled in the art can readily adapt currently available procedures to generate peptides, pharmaceutical agents and the like, capable of binding to a specific peptide sequence, in order to generate rationally designed antipeptide peptides, for example see Hurby et al., Application of Synthetic Peptides: Antisense Peptides," In Synthetic Peptides, A User's Guide, W.H. Freeman, NY (1992), pp. 289-307, and Kaspaczak et al., Biochemistry 28:9230-8 (1989), or pharmaceutical agents, or the like.

In addition to the foregoing, one class of agents of the present invention, as broadly described, can be used to control gene expression through binding to one of the ORFs or EMFs of the present invention. As described above, such agents can be randomly screened or rationally designed/selected. Targeting the ORF or EMF allows a skilled artisan to design sequence specific or element specific agents, modulating the

expression of either a single ORF or multiple ORFs which rely on the same EMF for expression control. One class of DNA binding agents are agents which contain base residues which hybridize or form a triple helix formation by binding to DNA or RNA. Such agents can be based on the classic phosphodiester, ribonucleic acid backbone, or 5 can be a variety of sulphydryl or polymeric derivatives which have base attachment capacity.

Agents suitable for use in these methods usually contain 20 to 40 bases and are designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 10 (1988); and Dervan et al., Science 251:1360 (1991)) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Triple helix-formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both 15 techniques have been demonstrated to be effective in model systems. Information contained in the sequences of the present invention is necessary for the design of an antisense or triple helix oligonucleotide and other DNA binding agents.

Agents which bind to a protein encoded by one of the ORFs of the present invention can be used as a diagnostic agent. Agents which bind to a protein encoded by 20 one of the ORFs of the present invention can be formulated using known techniques to generate a pharmaceutical composition.

## 5.16 USE OF NUCLEIC ACIDS AS PROBES

Another aspect of the subject invention is to provide for polypeptide-specific 25 nucleic acid hybridization probes capable of hybridizing with naturally occurring nucleotide sequences. The hybridization probes of the subject invention may be derived from any of the nucleotide sequences SEQ ID NO: 1-3 or 5. Because the corresponding gene is only expressed in a limited number of tissues, a hybridization probe derived from of any of the nucleotide sequences SEQ ID NO: 1-3 or 5 can be 30 used as an indicator of the presence of RNA of cell type of such a tissue in a sample.

Any suitable hybridization technique can be employed, such as, for example, *in situ* hybridization. PCR as described in US Patents Nos. 4,683,195 and 4,965,188 provides additional uses for oligonucleotides based upon the nucleotide sequences. Such probes used in PCR may be of recombinant origin, may be chemically synthesized, or a mixture of both. The probe will comprise a discrete nucleotide sequence for the detection of identical sequences or a degenerate pool of possible sequences for identification of closely related genomic sequences.

Other means for producing specific hybridization probes for nucleic acids include the cloning of nucleic acid sequences into vectors for the production of mRNA probes. Such vectors are known in the art and are commercially available and may be used to synthesize RNA probes *in vitro* by means of the addition of the appropriate RNA polymerase as T7 or SP6 RNA polymerase and the appropriate radioactively labeled nucleotides. The nucleotide sequences may be used to construct hybridization probes for mapping their respective genomic sequences. The nucleotide sequence provided herein may be mapped to a chromosome or specific regions of a chromosome using well known genetic and/or chromosomal mapping techniques. These techniques include *in situ* hybridization, linkage analysis against known chromosomal markers, hybridization screening with libraries or flow-sorted chromosomal preparations specific to known chromosomes, and the like. The technique of fluorescent *in situ* hybridization of chromosome spreads has been described, among other places, in Verma et al (1988) Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York NY.

Fluorescent *in situ* hybridization of chromosomal preparations and other physical chromosome mapping techniques may be correlated with additional genetic map data. Examples of genetic map data can be found in the 1994 Genome Issue of Science (265:1981f). Correlation between the location of a nucleic acid on a physical chromosomal map and a specific disease (or predisposition to a specific disease) may help delimit the region of DNA associated with that genetic disease. The nucleotide sequences of the subject invention may be used to detect differences in gene sequences between normal, carrier or affected individuals.

## 5.17 PREPARATION OF SUPPORT BOUND OLIGONUCLEOTIDES

Oligonucleotides, i.e., small nucleic acid segments, may be readily prepared by, for example, directly synthesizing the oligonucleotide by chemical means, as is commonly practiced using an automated oligonucleotide synthesizer.

5        Support bound oligonucleotides may be prepared by any of the methods known to those of skill in the art using any suitable support such as glass, polystyrene or Teflon. One strategy is to precisely spot oligonucleotides synthesized by standard synthesizers. Immobilization can be achieved using passive adsorption (Inouye & Hondo, 1990 *J. Clin Microbiol* 28(6) 1462-72); using UV light (Nagata *et al.*, 1985; Dahlen *et al.*, 1987;

10      Morrissey & Collins, *Mol. Cell Probes* 1989 3(2) 189-207) or by covalent binding of base modified DNA (Keller *et al.*, 1988; 1989); all references being specifically incorporated herein.

Another strategy that may be employed is the use of the strong biotin-streptavidin interaction as a linker. For example, Broude *et al.* (1994) *Proc. Natl. Acad. Sci USA* 91(8) 3072-6 describe the use of biotinylated probes, although these are duplex probes, that are immobilized on streptavidin-coated magnetic beads. Streptavidin-coated beads may be purchased from Dynal, Oslo. Of course, this same linking chemistry is applicable to coating any surface with streptavidin. Biotinylated probes may be purchased from various sources, such as, e.g., Operon Technologies (Alameda, CA).

20      Nunc Laboratories (Naperville, IL) is also selling suitable material that could be used. Nunc Laboratories have developed a method by which DNA can be covalently bound to the microwell surface termed CovaLink NH. CovaLink NH is a polystyrene surface grafted with secondary amino groups (> NH) that serve as bridge-heads for further covalent coupling. CovaLink Modules may be purchased from Nunc Laboratories. DNA molecules may be bound to CovaLink exclusively at the 5'-end by a phosphoramidate bond, allowing immobilization of more than 1 pmol of DNA (Rasmussen *et al.*, (1991) *Anal Biochem* 198(1) 138-42.

The use of CovaLink NH strips for covalent binding of DNA molecules at the 5'-end has been described (Rasmussen *et al.*, 1991). In this technology, a phosphoramidate bond is employed (Chu *et al.*, 1983 *Nucleic Acids* 11(18) 6513-29). This is beneficial as

immobilization using only a single covalent bond is preferred. The phosphoramidate bond joins the DNA to the CovaLink NH secondary amino groups that are positioned at the end of spacer arms covalently grafted onto the polystyrene surface through a 2 nm long spacer arm. To link an oligonucleotide to CovaLink NH via an phosphoramidate bond, the  
5 oligonucleotide terminus must have a 5'-end phosphate group. It is, perhaps, even possible for biotin to be covalently bound to CovaLink and then streptavidin used to bind the probes.

More specifically, the linkage method includes dissolving DNA in water (7.5 ng/ $\mu$ l) and denaturing for 10 min. at 95°C and cooling on ice for 10 min. Ice-cold 0.1 M  
10 1-methylimidazole, pH 7.0 (1-MeIm<sub>7</sub>), is then added to a final concentration of 10 mM 1-MeIm<sub>7</sub>. A ss DNA solution is then dispensed into CovaLink NH strips (75  $\mu$ l/well) standing on ice.

Carbodiimide 0.2 M 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), dissolved in 10 mM 1-MeIm<sub>7</sub>, is made fresh and 25  $\mu$ l added per well. The strips are  
15 incubated for 5 hours at 50°C. After incubation the strips are washed using, e.g., Nunc-Immuno Wash; first the wells are washed 3 times, then they are soaked with washing solution for 5 min., and finally they are washed 3 times (where in the washing solution is 0.4 N NaOH, 0.25% SDS heated to 50°C).

It is contemplated that a further suitable method for use with the present invention  
20 is that described in PCT Patent Application WO 90/03382 (Southern & Maskos), incorporated herein by reference. This method of preparing an oligonucleotide bound to a support involves attaching a nucleoside 3'-reagent through the phosphate group by a covalent phosphodiester link to aliphatic hydroxyl groups carried by the support. The oligonucleotide is then synthesized on the supported nucleoside and protecting groups  
25 removed from the synthetic oligonucleotide chain under standard conditions that do not cleave the oligonucleotide from the support. Suitable reagents include nucleoside phosphoramidite and nucleoside hydrogen phosphorate.

An on-chip strategy for the preparation of DNA probe for the preparation of DNA probe arrays may be employed. For example, addressable laser-activated  
30 photodeprotection may be employed in the chemical synthesis of oligonucleotides directly

on a glass surface, as described by Fodor *et al.* (1991) Science 251(4995) 767-73, incorporated herein by reference. Probes may also be immobilized on nylon supports as described by Van Ness *et al.* (1991) Nucleic Acids Res. 19(12) 3345-50; or linked to Teflon using the method of Duncan & Cavalier (1988) Anal Biochem 169(1) 104-8; all references being specifically incorporated herein.  
5

To link an oligonucleotide to a nylon support, as described by Van Ness *et al.* (1991), requires activation of the nylon surface via alkylation and selective activation of the 5'-amine of oligonucleotides with cyanuric chloride.

One particular way to prepare support bound oligonucleotides is to utilize the light-generated synthesis described by Pease *et al.*, (1994) Proc. Natl. Acad. Sci USA 91(11) 5022-6. These authors used current photolithographic techniques to generate arrays of immobilized oligonucleotide probes (DNA chips). These methods, in which light is used to direct the synthesis of oligonucleotide probes in high-density, miniaturized arrays, utilize photolabile 5'-protected *N*-acyl-deoxynucleoside phosphoramidites, surface linker chemistry and versatile combinatorial synthesis strategies. A matrix of 256 spatially defined oligonucleotide probes may be generated in this manner.  
10  
15

### **5.18 PREPARATION OF NUCLEIC ACID FRAGMENTS**

The nucleic acids may be obtained from any appropriate source, such as cDNAs, genomic DNA, chromosomal DNA, microdissected chromosome bands, cosmid or YAC inserts, and RNA, including mRNA without any amplification steps. For example, Sambrook *et al.* (1989) describes three protocols for the isolation of high molecular weight DNA from mammalian cells (p. 9.14-9.23).  
20

DNA fragments may be prepared as clones in M13, plasmid or lambda vectors and/or prepared directly from genomic DNA or cDNA by PCR or other amplification methods. Samples may be prepared or dispensed in multiwell plates. About 100-1000 ng of DNA samples may be prepared in 2-500 ml of final volume.  
25

The nucleic acids would then be fragmented by any of the methods known to those of skill in the art including, for example, using restriction enzymes as described at 9.24-9.28 of Sambrook *et al.* (1989), shearing by ultrasound and NaOH treatment.

Low pressure shearing is also appropriate, as described by Schriefer *et al.* (1990) Nucleic Acids Res. 18(24) 7455-6. In this method, DNA samples are passed through a small French pressure cell at a variety of low to intermediate pressures. A lever device allows controlled application of low to intermediate pressures to the cell. The results of 5 these studies indicate that low-pressure shearing is a useful alternative to sonic and enzymatic DNA fragmentation methods.

One particularly suitable way for fragmenting DNA is contemplated to be that using the two base recognition endonuclease, *CviJI*, described by Fitzgerald *et al.* (1992) Nucleic Acids Res. 20(14) 3753-62. These authors described an approach for the rapid 10 fragmentation and fractionation of DNA into particular sizes that they contemplated to be suitable for shotgun cloning and sequencing.

The restriction endonuclease *CviJI* normally cleaves the recognition sequence PuGCPy between the G and C to leave blunt ends. Atypical reaction conditions, which alter the specificity of this enzyme (*CviJI\*\**), yield a quasi-random distribution of DNA 15 fragments from the small molecule pUC19 (2688 base pairs). Fitzgerald *et al.* (1992) quantitatively evaluated the randomness of this fragmentation strategy, using a *CviJI\*\** digest of pUC19 that was size fractionated by a rapid gel filtration method and directly ligated, without end repair, to a lac Z minus M13 cloning vector. Sequence analysis of 76 clones showed that *CviJI\*\** restricts pyGCPy and PuGCPu, in addition to PuGCPy sites, 20 and that new sequence data is accumulated at a rate consistent with random fragmentation.

As reported in the literature, advantages of this approach compared to sonication and agarose gel fractionation include: smaller amounts of DNA are required (0.2-0.5 ug instead of 2-5 ug); and fewer steps are involved (no preligation, end repair, chemical extraction, or agarose gel electrophoresis and elution are needed).

25 Irrespective of the manner in which the nucleic acid fragments are obtained or prepared, it is important to denature the DNA to give single stranded pieces available for hybridization. This is achieved by incubating the DNA solution for 2-5 minutes at 80-90°C. The solution is then cooled quickly to 2°C to prevent renaturation of the DNA fragments before they are contacted with the chip. Phosphate groups must also be 30 removed from genomic DNA by methods known in the art.

## **5.19 PREPARATION OF DNA ARRAYS**

Arrays may be prepared by spotting DNA samples on a support such as a nylon membrane. Spotting may be performed by using arrays of metal pins (the positions of which correspond to an array of wells in a microtiter plate) to repeated by transfer of about 20 nl of a DNA solution to a nylon membrane. By offset printing, a density of dots higher than the density of the wells is achieved. One to 25 dots may be accommodated in 1 mm<sup>2</sup>, depending on the type of label used. By avoiding spotting in some preselected number of rows and columns, separate subsets (subarrays) may be formed. Samples in one subarray may be the same genomic segment of DNA (or the same gene) from different individuals, or may be different, overlapped genomic clones. Each of the subarrays may represent replica spotting of the same samples. In one example, a selected gene segment may be amplified from 64 patients. For each patient, the amplified gene segment may be in one 96-well plate (all 96 wells containing the same sample). A plate for each of the 64 patients is prepared. By using a 96-pin device, all samples may be spotted on one 8 x 12 cm membrane. Subarrays may contain 64 samples, one from each patient. Where the 96 subarrays are identical, the dot span may be 1 mm<sup>2</sup> and there may be a 1 mm space between subarrays.

Another approach is to use membranes or plates (available from NUNC, Naperville, Illinois) which may be partitioned by physical spacers e.g. a plastic grid molded over the membrane, the grid being similar to the sort of membrane applied to the bottom of multiwell plates, or hydrophobic strips. A fixed physical spacer is not preferred for imaging by exposure to flat phosphor-storage screens or x-ray films.

The present invention is illustrated in the following examples. Upon consideration of the present disclosure, one of skill in the art will appreciate that many other embodiments and variations may be made in the scope of the present invention.

Accordingly, it is intended that the broader aspects of the present invention not be limited to the disclosure of the following examples. The present invention is not to be limited in scope by the exemplified embodiments which are intended as illustrations of single aspects of the invention, and compositions and methods which are functionally equivalent are within the scope of the invention. Indeed, numerous modifications and variations in the

practice of the invention are expected to occur to those skilled in the art upon consideration of the present preferred embodiments. Consequently, the only limitations which should be placed upon the scope of the invention are those which appear in the appended claims.

5 All references cited within the body of the instant specification are hereby incorporated by reference in their entirety.

## 6.0 EXAMPLES

### EXAMPLE 1

#### Isolation of SEQ ID NO: 1 from a cDNA Library of Thymus

10

A plurality of novel nucleic acids were obtained from a cDNA library prepared from thymus (Hyseq clone identification numbers 16395938 (SEQ ID NO: 1)) using standard PCR, sequencing by hybridization sequence signature analysis, and Sanger sequencing techniques. The inserts of the library were amplified with PCR using primers specific for vector sequences flanking the inserts. These samples were spotted onto nylon membranes and interrogated with oligonucleotide probes to give sequence signatures. The clones were clustered into groups of similar or identical sequences, and single representative clones were selected from each group for gel sequencing. The 5' sequence of the amplified inserts was then deduced using the reverse M13 sequencing primer in a typical Sanger sequencing protocol. PCR products were purified and subjected to fluorescent dye terminator cycle sequencing. Single-pass gel sequencing was done using a 377 Applied Biosystems (ABI) sequencer. The insert was identified as a novel sequence not previously obtained from this library and not previously reported in public databases. The sequence was designated as SEQ ID NO: 1.

25

EXAMPLE 2  
ASSEMBLAGE OF SEQ ID NO: 2

The nucleic acid of the present invention, designated as SEQ ID NO: 2 was  
5 assembled using SEQ ID NO:1 as a seed. Then a recursive algorithm was used to extend  
the seed into an extended assemblage, by pulling additional sequences from different  
databases (i.e., Hyseq's database containing EST sequences, dbEST version 114, gb pri  
114, and UniGene version 101) that belong to this assemblage. The algorithm terminated  
when there was no additional sequences from the above databases that would extend the  
10 assemblage. Inclusion of component sequences into the assemblage was based on a  
BLASTN hit to the extending assemblage with BLAST score greater than 300 and percent  
identity greater than 95%..

The nearest neighbor result for the assembled contigs were obtained by a FASTA  
version 3 search against Genpept release 114, using FASTXY algorithm. FASTXY is an  
15 improved version of FASTA alignment which allows in-codon frame shifts. The nearest  
neighbor result showed the closest homologue for each assemblage from Genpept (and  
contains the translated amino acid sequences for which the assemblage encodes). The  
nearest neighbor results is set forth below:

SEQ ID NO:	Accession No.	Description	Smith-Waterman Score	% Identity
2	Z24680	Homo sapiens Garp	318	46.212

20

A polypeptide was predicted to be encoded by SEQ ID NO: 2 as set forth below.  
The polypeptide was predicted using a software program called FASTY (available from  
25 <http://fasta.bioch.virginia.edu>) which selects a polypeptide based on a comparison of  
translated novel polynucleotide to known polypeptides (W.R. Pearson, Methods in  
Enzymology, 183: 63-98 (1990), herein incorporated by reference).

Predicted beginning nucleotide location corresponding to first amino acid residue of amino acid segment	Predicted end nucleotide location corresponding to last amino acid residue of amino acid segment	AMINO ACID ENCODED BY SEQ ID NO: 2 (A=Alanine, C=Cysteine, D=Aspartic Acid, E=Glutamic Acid, F=Phenylalanine, G=Glycine, H=Histidine, I=Isoleucine, K=Lysine, L=Leucine, M=Methionine, N=Asparagine, P=Proline, Q=Glutamine, R=Arginine, S=Serine, T=Threonine, V=Valine, W=Tryptophan, Y=Tyrosine, X=Unknown, *=Stop Codon, / =possible nucleotide deletion, \=possible nucleotide insertion)
20	383	VSLAGNTIMRLDDSVFEGLERLRELDLQRNYIFEIE GGAFDGLAELRHLNLAFNNLPCIVDFGLTRLRVLN VSYNVLEWFLATGGEAAFELETLDLSHNQLFFPL LPQYSKLRTLLRDN (SEQ ID NO: 18)

5

### EXAMPLE 3

#### ASSEMBLAGE OF SEQ ID NOs: 3 and 4

Assembly of novel nucleotide sequence of SEQ ID NO: 3 was accomplished by using an EST sequence SEQ ID NO: 1 as a seed. The seed was extended by gel sequencing (377 Applied Biosystems (ABI) sequencer) using primers to extend the 3' end (primer extension). The 5' end was extended using RACE, as disclosed in Marathon-Ready™ cDNA User Manual (PT1156-1) (Clontech) herein incorporated by reference.

A polypeptide (SEQ ID NO:4) was predicted to be encoded by SEQ ID NO:3 as set forth below. The polypeptide was predicted using a software program called BLASTX which selects a polypeptide based on a comparison of translated novel

polynucleotide to known polynucleotides. The initial methionine starts at position 168 of SEQ ID NO:3 and the putative stop codon, TGA, begins at position 2244 of the nucleotide sequence.

Figure 1A and Figure 1B show the BLASTX amino acid sequence alignment between the protein encoded by SEQ ID NO: 3 (i.e. SEQ ID NO: 4) LRR protein-like polypeptide and human Garp protein precursor (Garpin) SEQ ID NO: 19, indicating that the two sequences share 51% similarity over 637 amino acid residues of SEQ ID NO: 4 and 38% identity over the same 637 amino acid residues of SEQ ID NO: 4.

Figure 2 shows the BLASTX amino acid sequence alignment between the protein encoded by SEQ ID NO: 3 (i.e. SEQ ID NO: 4) LRR protein-like polypeptide and human insulin-like growth factor binding protein complex acid labile chain precursor protein SEQ ID NO: 20, indicating that the two sequences share 44% similarity over 510 amino acid residues of SEQ ID NO: 4 and 30% identity over the same 510 amino acid residues of SEQ ID NO: 4.

Figure 3 shows the BLASTX amino acid sequence alignment between the protein encoded by SEQ ID NO: 3 (i.e. SEQ ID NO: 4) LRR protein-like polypeptide and human glycoprotein V protein SEQ ID NO: 21, indicating that the two sequences share 45% similarity over 386 amino acid residues of SEQ ID NO: 4 and 32% identity over the same 386 amino acid residues of SEQ ID NO: 4.

Figure 4 shows the BLASTX amino acid sequence alignment between the protein encoded by SEQ ID NO: 3 (i.e. SEQ ID NO: 4) LRR protein-like polypeptide and synthetic WD-40 domain containing insulin-like growth factor binding protein SEQ ID NO: 22, indicating that the two sequences share 44% similarity over 467 amino acid residues of SEQ ID NO: 4 and 30% identity over the same 467 amino acid residues of SEQ ID NO: 4

A predicted approximately eighteen-residue signal peptide is encoded from approximately residue 1 through residue 18 of SEQ ID NO: 4 (SEQ ID NO: 15). The extracellular portion is useful on its own. This can be confirmed by expression in mammalian cells and sequencing of the cleaved product. The signal peptide region was predicted using the Kyte-Doolittle hydrophobicity prediction algorithm (J. Mol Biol,

157, pp. 105-31 (1982), incorporated herein by reference). One of skill in the art will recognize that the actual cleavage site may be different than that predicted by the computer program.

A predicted approximately twenty six-residue transmembrane region peptide is  
5 encoded from approximately residue 648 through residue 673 of SEQ ID NO: 4 (SEQ ID NO: 16). The transmembrane portion is useful on its own. This can be confirmed by expression in mammalian cells. The transmembrane peptide region was predicted using the Kyte-Doolittle hydrophobicity prediction algorithm (J. Mol Biol, 157, pp. 105-31 (1982), incorporated herein by reference). One of skill in the art will recognize  
10 that the actual cleavage site may be different than that predicted by the computer program.

Using eMATRIX software package (Stanford University, Stanford, CA) (Wu et al., J. Comp. Biol., vol. 6, pp. 219-235 (1999), herein incorporated by reference), LRR protein-like polypeptide is expected to have nine leucine-rich repeat signatures within  
15 residues 378-391, 134-147, 535-548, 375-388, 252-265, 225-238, 560-573, 351-364, and 180-193 of SEQ ID NO: 4 (SEQ ID NO: 6-14). The domains corresponding to SEQ ID NO: 6-14 are as follows wherein A=Alanine, C=Cysteine, D=Aspartic Acid, E=Glutamic Acid, F=Phenylalanine, G=Glycine, H=Histidine, I=Isoleucine, K=Lysine, L=Leucine, M=Methionine, N=Asparagine, P=Proline, Q=Glutamine, R=Arginine,  
20 S=Serine, T=Threonine, V=Valine, W=Tryptophan, Y=Tyrosine:

Leucine-rich repeat signature

LTELDSLHNQLSEL

designated as SEQ ID NO: 6 p-value of 5.5e-15, PR00019A (identification number correlating to signature); located at residues 378-391 of SEQ ID NO: 4,

25 Leucine-rich repeat signature

LRRLDLSGNALTED

designated as SEQ ID NO: 7 p-value of 3.739e-10, PR00019A (identification number correlating to signature); located at residues 134-147 of SEQ ID NO: 4,

Leucine-rich repeat signature

30 FGNLRDLDLSGNCL

[REDACTED]

designated as SEQ ID NO: 8 p-value of 1.0e-9, PR00019A (identification number correlating to signature); located at residues 535-548 of SEQ ID NO: 4,  
Leucine-rich repeat signature  
PGALTELDLSHNQL  
5 designated as SEQ ID NO: 9 p-value of 2.44e-9, PR00019A (identification number correlating to signature); located at residues 375-388 of SEQ ID NO: 4,  
Leucine-rich repeat signature  
LETLDLSHNQLLFF  
designated as SEQ ID NO: 10 p-value of 3.333e-9, PR00019A (identification number correlating to signature); located at residues 252-265 of SEQ ID NO: 4,  
10 Leucine-rich repeat signature  
LTRLRVLNVSYNVL  
designated as SEQ ID NO: 11 p-value of 4.96e-9, PR00019A (identification number correlating to signature); located at residues 225-238 of SEQ ID NO: 4,  
15 Leucine-rich repeat signature  
LETLDLRRNSLTAL  
designated as SEQ ID NO: 12 p-value of 7.0e-9, PR00019A (identification number correlating to signature); located at residues 560-573 of SEQ ID NO: 4,  
Leucine-rich repeat signature  
20 MPSLSHNLHQNCL  
designated as SEQ ID NO: 13 p-value of 7.84e-9, PR00019A (identification number correlating to signature); located at residues 351-364 of SEQ ID NO: 4,  
Leucine-rich repeat signature  
LERLRELDLQRNYI  
25 designated as SEQ ID NO: 14 p-value of 9.64e-0, PR00019A (identification number correlating to signature); located at residues 180-193 of SEQ ID NO: 4.

#### EXAMPLE 4

30 A. Expression of SEQ ID NO: 4 in cells

Chinese Hamster Ovary (CHO) cells or other suitable cell types are grown in DMEM (ATCC) and 10% fetal bovine serum (FBS) (Gibco) to 70% confluence. Prior to transfection the media is changed to DMEM and 0.5% FCS. Cells are transfected with cDNAs for SEQ ID NO: 3 or 5 or with pBGal vector by the FuGENE-6 transfection reagent (Boehringer). In summary, 4  $\mu$ l of FuGENE-6 is diluted in 100  $\mu$ l of DMEM and incubated for 5 minutes. Then, this is added to 1  $\mu$ g of DNA and incubated for 15 minutes before adding it to a 35 mm dish of CHO cells. The CHO cells are incubated at 37°C with 5% CO<sub>2</sub>. After 24 hours, media and cell lysates are collected, centrifuged and dialyzed against assay buffer (15 mM Tris pH 7.6, 134 mM NaCl, 5 mM glucose, 3 mM CaCl<sub>2</sub> and MgCl<sub>2</sub>).

**B. Expression Study Using SEQ ID NO: 1-3 or 5**

The expression of SEQ ID NO: 1-3 or 5 in various tissues is analyzed using a semi-quantitative polymerase chain reaction-based technique. Human cDNA libraries are used as sources of expressed genes from tissues of interest (adult bladder, adult brain, adult heart, adult kidney, adult lymph node, adult liver, adult lung, adult ovary, adult placenta, adult rectum, adult spleen, adult testis, bone marrow, thymus, thyroid gland, fetal kidney, fetal liver, fetal liver-spleen, fetal skin, fetal brain, fetal leukocyte and macrophage). Gene-specific primers are used to amplify portions of the SEQ ID NO: 1-3 or 5 sequence from the samples. Amplified products are separated on an agarose gel, transferred and chemically linked to a nylon filter. The filter is then hybridized with a radioactively labeled (<sup>33</sup>P-dCTP) double-stranded probe generated from SEQ ID NO: 1-3 or 5 using a Klenow polymerase, random-prime method. The filters are washed (high stringency) and used to expose a phosphorimaging screen for several hours. Bands indicate the presence of cDNA including SEQ ID NO: 1-3 or 5 sequences in a specific library, and thus mRNA expression in the corresponding cell type or tissue.

CLAIMS

WE CLAIM:

5           1. An isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 2-3 and 5, the translated protein coding portion thereof, the mature protein coding portion thereof, the extracellular portion thereof, or the active domain thereof.

10          2. An isolated polynucleotide encoding a polypeptide with biological activity, which polynucleotide hybridizes to the complement of a polynucleotide of claim 1 under stringent hybridization conditions.

15          3. An isolated polynucleotide encoding a polypeptide with biological activity, said polynucleotide having greater than about 90% sequence identity with the polynucleotide of claim 1.

20          4. The polynucleotide of claim 1 which is a DNA sequence.

25          5. An isolated polynucleotide which comprises the complement of the polynucleotide of claim 1.

              6. A vector comprising the polynucleotide of claim 1.

              7. An expression vector comprising the polynucleotide of claim 1.

              8. A host cell genetically engineered to express the polynucleotide of claim 1.

9. The host cell of claim 8 wherein the polynucleotide is in operative association with a regulatory sequence that controls expression of the polynucleotide in the host cell.

5 10. An isolated polypeptide comprising an amino acid sequence which is at least 80% identical to the amino acid sequence selected from the group consisting of SEQ ID NO: 4, 6-17 and 18, the translated protein coding portion thereof, the mature protein coding portion thereof, the extracellular portion thereof, or the active domain thereof.

10 11. A composition comprising the polypeptide of claim 10 and a carrier.

15 12. A polypeptide, having LRR protein-like activity, comprising at least ten consecutive amino acids from the polypeptide sequences selected from the group consisting of SEQ ID NO: 4, 6-17 and 18.

20 13. The polypeptide of claim 12, comprising at least five consecutive amino acids from the polypeptide sequences selected from the group consisting of SEQ ID NO. 4, 6-17 and 18.

14. A polynucleotide encoding a polypeptide according to claim 12.

15. A polynucleotide encoding a polypeptide according to claim 13.

25 16. A polynucleotide encoding a polypeptide according to claim 10.

17. An antibody specific for the polypeptide of claim 10.

18. A method for detecting the polynucleotide of claim 1 in a sample, comprising:

a) contacting the sample with a compound that binds to and forms a complex with the polynucleotide of claim 1 for a period sufficient to form the complex;

5 and

b) detecting the complex, so that if a complex is detected, the polynucleotide of claim 1 is detected.

19. A method for detecting the polynucleotide of claim 1 in a sample, comprising:

a) contacting the sample under stringent hybridization conditions with nucleic acid primers that anneal to the polynucleotide of claim 1 under such conditions;

b) amplifying a product comprising at least a portion of the polynucleotide of claim 1; and

c) detecting said product and thereby the polynucleotide of claim 1 in the sample.

20. The method of claim 19, wherein the polynucleotide comprises an RNA molecule and the method further comprises reverse transcribing an annealed RNA molecule into a cDNA polynucleotide.

21. A method for detecting the polypeptide of claim 10 in a sample, comprising:

a) contacting the sample with a compound that binds to and forms a complex with the polypeptide under conditions and for a period sufficient to form the complex; and

b) detecting formation of the complex, so that if a complex formation is detected, the polypeptide of claim 10 is detected.

30

22. A method for identifying a compound that binds to the polypeptide of claim 10, comprising:

- a) contacting the compound with the polypeptide of claim 10 under conditions and for a time sufficient to form a polypeptide/compound complex; and
- 5 b) detecting the complex, so that if the polypeptide/compound complex is detected, a compound that binds to the polypeptide of claim 10 is identified.

23. A method for identifying a compound that binds to the polypeptide of claim 10, comprising:

- 10 a) contacting the compound with the polypeptide of claim 10, in a cell, for a time sufficient to form a polypeptide/compound complex, wherein the complex drives expression of a reporter gene sequence in the cell; and
- b) detecting the complex by detecting reporter gene sequence expression, so that if the polypeptide/compound complex is detected, a compound that binds to the polypeptide of claim 10 is identified.

24. A method of producing an LRR protein-like polypeptide, comprising,

- 20 a) culturing the host cell of claim 8 under conditions sufficient to express the polypeptide in said cell; and
- b) isolating the polypeptide from the cell culture or cells of step (a).

25. A kit comprising the polypeptide of claim 10.

26. A nucleic acid array comprising the polynucleotide of claim 1 or a unique segment of the polynucleotide of claim 1 attached to a surface.

27. The array of claim 26, wherein the array detects full-matches to the polynucleotide or a unique segment of the polynucleotide of claim 1.

28. The array of claim 26, wherein the array detects mismatches to  
the polynucleotide or a unique segment of the polynucleotide of claim 1.

29. A method of treatment of a subject in need of enhanced activity  
5 or expression of LRR protein-like polypeptide of claim 10 comprising administering  
to the subject a composition selected from the group consisting of:

(a) a therapeutic amount of a agonist of said polypeptide;  
(b) a therapeutic amount of the polypeptide; and  
(c) a therapeutic amount of a polynucleotide encoding the  
10 polypeptide in a form and under conditions such that the  
polypeptide is produced,

and a pharmaceutically acceptable carrier.

30. A method of treatment of a subject having need to inhibit activity  
15 or expression of LRR protein-like polypeptide of claim 10 comprising administering  
to the subject a composition selected from the group consisting of:

(a) a therapeutic amount of an antagonist to said polypeptide;  
(b) a therapeutic amount of a polynucleotide that inhibits the  
expression of the nucleotide sequence encoding said  
20 polypeptide; and  
(c) a therapeutic amount of a polypeptide that competes with the  
LRR protein-like polypeptide for its ligand

and a pharmaceutically acceptable carrier.

25

## ABSTRACT

The invention provides novel polynucleotides and polypeptides encoded by such  
5 polynucleotides and mutants or variants thereof that correspond to a novel human  
secreted LRR protein-like polypeptide. These polynucleotides comprise nucleic acid  
sequences isolated from cDNA library from thymus (Hyseq clone identification  
numbers 16395938 (SEQ ID NO: 1)). Other aspects of the invention include vectors  
containing processes for producing novel human secreted LRR protein-like  
10 polypeptides, and antibodies specific for such polypeptides.

**BLASTX ALIGNMENT OF SEQ ID NO: 4 LEUCINE-RICH REPEAT PROTEIN-LIKE POLYPEPTIDE (IDENTIFIED AS LRR PROTEIN-LIKE) WITH HUMAN GARP PROTEIN PRECURSOR PROTEIN SEQ ID NO: 19**

---

Query: Leucine-rich repeat-like protein (SEQ ID 4)  
Sbjct: Human Garp protein precursor protein gi|5031707 ref|NP\_005503.1| glycoprotein A repetitions predominant precursor; garpin >sp|Q14392|GARP\_HUMAN GARP PROTEIN PRECURSOR (GARPIN) >pir||S42799 garp [Homo sapiens] (SEQ ID NO: 19)  
Length = 662

Score = 822 (289.4 bits), Expect = 4. 9e-81, P = 4. 9e-81  
Identities = 245/637 (38%), Positives = 328/637 (51%)

Query: 267 CKLVGGAAADCRCGQSLASVPPSLIPPHARMLTIDANPLKTIWNHSLQDPYPLLESLSLHSCHL 446  
Sbjct: 26 CRMVKVSKVSCQVLQVPSVLPDPDTEITDLSGNQLRSILASPLGVYTAIRHLDSTNEI 85

Query: 447 ERISRGAFQEOQGHRLSIVLIGNDNCILSENYEETAAALHALPGLRRLDUGNIALML 626  
Sbjct: + GAFQ + HI L L N L+ +A L IP + LDISGN+L + +L  
86 SFLQPGFAFOQALTHLEHLHSLAHNRLAMATAISAGGLGPIPRVISLDISGNLYSGELL 145

Query: 627 QNLSSLRVSLSLAGNITMRIDDSSVFEGLERLRELDLQRNYIFIEEGAFDGLAELRHNL 806  
Sbjct: SL ++SLA N++ RL F + L + LDL N + +TE GAF+GL L HNL+ N+L C1 DF L + LRVL++S N +E F A+ +A F+L LDL N+LL FP L 205

Query: 807 FNNLPCITVDFGLTRLRLVNVSYNVLWLF-LATGGEAERFLETDLISHNOLLEFFPLRQYS 983  
Sbjct: 206 RNSLTCISDFSLQQLRVLIDLSCNSNTEAFQATASQPOAEFQLTWILDIRENKLHFPDLAALP 265

Query: 984 KIRTLLILRDNNMGMFYRDLIYNTSSPREMVAQFFLVDG-NVNTINITYSLSWEFSSSDLADR 1160  
Sbjct: +L L L NN+ R T P++ +G + ++ S S L+ L  
266 RLIYINTL-SNNL--IR--LPTGPPQDSKGIIHAPSEGWSALPLSAPS-GNASGRPLSQL 318

Query: 1161 FLDMSQNQFOYLIPDGFLRKMPMSLISHNLHQNCLMLTHIREHEPPGALTIEDLSHQSEL 1340  
Sbjct: 319 NLDLSYNEIELIPDSFLEHITSLCHFLNLSRNCLRTFEARRLSLPCIMLIDLSHNAETL 378

Query: 1341 HLIAPGLIASCTIGSIRLFLNLSNNQNLIGVPPGIFANARNLTTLDSHNOISLCPLPAASDRV 1520  
Sbjct: L A LGSLR L N L +PP FAN ++ L+ + N+S C P D G 432  
379 ELG--ARAIGSIRTLLIQLQMGNAIRDLPYTFANIASLQRNLIQGVSVPCGGP--DEPG

**Fig. 1A**

**BLASTX ALIGNMENT OF SEQ ID NO: 4 LEUCINE-RICH REPEAT PROTEIN-LIKE POLYPEPTIDE  
(IDENTIFIED AS LRR PROTEIN-LIKE) WITH HUMAN GARP PROTEIN PRECURSOR PROTEIN SEQ  
ID NO: 19**

---

Query: 1521 PPSCVDPRNMASLRSLSLEGCGIGALPDCPFQGTSILTYLDLSSNWG--VILNGSLAPLQDV 1694  
P CV F + SIRSLSL + L F T LT LDLSN G V G+I L+ 490  
Sbjct: 433 PSCCVAFSGITSRLSLSLVDDNEIELLRAGAFLHTPLTLEJLISSNPGLEVATGALGGLE-- 490  
Query: 1695 APMIQLVISLRNMGLHSSFMALDFSGFGNLRLDLSGNCLPFPFRCGSLAETLIDRNS 1874  
L+VIL+L+ GL + +D F L+ L+L+ N L P + +++LE IIDR NS  
Sbjct: 491 -ASLEVIALQNGNL-MVLQVQLPCFCICLKRINTLAENFLSHLPWTQAVSLEVIDLRNS 547  
Query: 1875 ITALPKQAVSEQLSRGLRTIYLISQNPDCCGVDGWGAHQ-H-GOTVADWAM-VTCNLSSK 2045  
+ LP A+ L LR +YL NP CCG +GW A Q H G+ D + C SS+  
Sbjct: 548 FSLLPGSAMGG-LETSIRRLLYIQLGNPLSCCG-NGWLAQHLQGRVNDVATQDLICRESSQ 605  
Query: 2046 IIRVTELPGGVPRDCCKWERL-DIGIILY-LVWLIPSC-LTILVACTVI 2177  
L P DC+ L + L+ L IL S LT L AC +  
Sbjct: 606 --EEVSLSHVRPEDCEKGGLKNINLITLTFLIVSAILITLIAACCV 651

**FIG. 1B**

BLASTX ALIGNMENT OF SEQ ID NO: 4 LEUCINE-RICH REPEAT PROTEIN-LIKE POLYPEPTIDE (IDENTIFIED AS LRR PROTEIN-LIKE) WITH HUMAN INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN COMPLEX ACID LABILE SUBUNIT PROTEIN SEQ ID NO: 20

Query: Leucine-rich repeat-like protein (SEQ ID 4)  
Sbjct: Human SP|002833|ALS\_PAPPA insulin-like growth factor binding protein complex acid labile chain precursor (ALS)  
>pir|Jc5239 insulin-like growth factor acid-labile chain - baboon (SEQ ID NO: 20)  
Length = 605

Score = 294 (103.5 bits), Expect = 1.1e-21, P = 1.1e-211  
Identities = 158/510 (30%), Positives = 228/510 (44%)

Query: /8  
KRSAALEHAGPGDDAPREPPAAGAA+IDMEL--LPLWLCLGFHFLL 212  
E G PG+ P A + N R I D P

Query: 213 TWGWRNRSGTATAASQGVCKIVGGADCRGOSIASV-PSSIPPHARM--ITIDANPIKTTI 383

Subjct: 80 WLDNNLSSIPPAAFRNLISSLA- FLNLOGGQLGSLEPQALLIGLENLCHHLERNQLRSL 137

Query: 384 WNHSLQPYPLLESLSIHSHCILRISRGAFQEQGHLRSIVLGDNCLISENYEETAALHALP 563  
 + P L L L + L R+ G F+ G+L L LG N L+ + AA L

Query: 564 GIRRDLGNSALTEDMAALMONTISSIRVSLAGNTIMRIDSVEFGIERPRTDOPNY 742  
150 AVGULFAILFALLUGLUSNKLRSKJEDGLREGJGNLWDNLGNWNSLAVID---AAGRGLG 194

Subjct: 195 GILRL L<sup>L+GN</sup> L<sup>L+</sup> LR + L<sup>L+</sup> N + + + VF L R1+L L<sup>RN</sup> GLRELVLAGNRLLAYLQPALF-SGLAELRELDLSRNALRAIKANVFAQQLRLOKLYIDRN 253

Query: 744 IFEIEGGAFDGLAELRHINLAFNNLPCITVD--F--GLTRLRVLVNVSYNVLLEMFLATGGEAA 914

Query: 234 IAAVAPGAFIUGLKALRMRWLDLSHNRVAGILEDTEPGLLGLRVRLSHNAASLRPRTEFDL 313  
915 FEELTDLISHNOL--LEPFILPOYSKRTTJIRDNNGCFYPT VNTSDEDEMAYAOT<sup>TT</sup> TV 1000

Subjct: 314 HFLLELQIGHNRIRQLAERSFEGLGQLEVLTLDHNO-----LOENV----VGAFLGLT 362

Query: 1086 DGNVNNTTIVSLWE--EFFSSDIADIRFLDMSQNQFOYLPDGLRKMPPLSILHQNCL 1255  
+ V N++ L E L I L + + + + + T I T I N T

sbjct: 423 VGEIEEQSLWGLAELLELDLTSNQLT--HLPHQFLFGIGKLEYLLLSHNRALAEPLPADALGP 480

Query: 1440 ARNITTLDSMSHNOQISLCPPIAASDRVGPSPCVDFRNMASLRSLSLEGCGIGAL 159  
Sbjct: + LD+SHN++ P + +G ++ RN SLR+ + + GL L  
481 LQRFAFLDVSHNRLEAPGSILAS -LGRRLRYLNLRNN -SLRTFTPOPPGDERL 531

FIG. 2

**BLASTX ALIGNMENT OF SEQ ID NO: 4 LEUCINE-RICH REPEAT PROTEIN-LIKE POLYPEPTIDE  
(IDENTIFIED AS LRR PROTEIN-LIKE) WITH HUMAN GLYCOPROTEIN V PROTEIN SEQ ID NO:  
21**

---

```

Query: Leucine-rich repeat-like protein (SEQ ID 4)
Sbjct: gi|4758460 ref|NP_004479.1| glycoprotein V (platelet) >sp|P40197|GPV_HUMAN PLATELET GLYCOPROTEIN V PRECURSOR (GPV)
(CD42D) >pir|A60164 platelet membrane glycoprotein V precursor - human >pir|A47507 platelet membrane glycoprotein V precursor [Homo sapiens] >gb|AAA03069.1| (L11238)
precursor - human emb|CAA80637.1| (Z23091) platelet glycoprotein V precursor [Homo sapiens] (SEQ ID NO: 21)
platelet membrane glycoprotein V [Homo sapiens] (SEQ ID NO: 21)
length = 560

Score = 273 (96.1 bits), Expect = 2.8e-20, Sum P(2) = 2.8e-20
Identities = 127/386 (32%), Positives = 177/386 (45%)

Query: 837 GLTRLRVIANSYNLEWFLATGGEA-AFELLETDLISHNQLLFFP-LLPQYSKRLTLLR 1007
Sbjct: 72 GMTVLQRIMSDSHIS-AVAPGTFSDLIKLKTRLSRKITHLPGALDKMVVLEQLFL-129
Query: 1008 DNNMGMFYRDLYNTSSPREMVAQFLIVDGNTVNITTVSI-WEEFSSSDLADLRFLDMSQNQF 1187
Sbjct: 130 D+N R + + + Q L ++ N + S+ + + L + L+ LD+S N
DHNA - -LRGIDQNMFQKLVLNLQELAINQNQDFLPASIF-----TNLENKLUDLSGNL 182
Sbjct: 138 QYLPGDFLRLKMPSLSHNLHONCIMLHIREHEPPGALTELDLISHNQLOSELHLAPGLASC 1367
Query: 1188 +LP G L L L LH N L++L GAITEL N + + APG
THLPKGLLGAQAKLERLILHHSNRIVSILDGGHINSIGALTEQFHRRHRSI--APGAFDR 240
Sbjct: 183

Query: 1368 LGSLRLFNLSSNQQLGVPPGLEANARNITTLDMSHNOISLCPLAASDRVGPSCVDFRN 1547
Sbjct: 241 L + L LS N L + P LF ++ N+T L + N L LP V F
LPNLSSLTLSRNHLAFLPSALFLHSHNLTLTLPENP--LAELPG-----VLFGE 288
Sbjct: 289 M ASLRSLSLEGCGGLALPDCPFQGTS-LTYLD--LSSNGWVIL-NGSLAPLQDVAPMLQV 1715
L + L L L LP F+ S L YL LS L G+ L + LQVL
MGGLQELWLNRTQLRTLPAAAFRNTISRLRYLIGVTISPRISALPOGAFOQGLGE---LQVL 344
Sbjct: 345

Query: 1716 SLRNMGHLHSSFMALDFSGFGNIRLDLDSGNCLIPIFPR--FGGSALAEETDLRRNSLTALP 1889
Sbjct: 345 +L + GL + L G G LR + L N L PR F + L E+ + L N L LP
ALHSNGLTLALPDGL-LRGIGKLRQVSLLRNRLRALPRAFLRNLISSIESVOLDHNQLETLP 403
Sbjct: 345

Query: 1890 QKAVSEQLSRLGRLRTIYLSQLNPYDC-CGVD---GWGALQH 1994
Sbjct: 404 GD-VFGALPR-LTEVLLGHNSWRCDCGLGPFLLGW-LRQH 439

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**FIG. 3**

**BLASTX ALIGNMENT OF SEQ ID NO: 4 LEUCINE-RICH REPEAT PROTEIN-LIKE POLYPEPTIDE  
(IDENTIFIED AS LRR PROTEIN-LIKE) WITH WD-40 DOMAIN CONTAINING INSULIN-LIKE  
GROWTH FACTOR BINDING PROTEIN SEQ ID NO: 22**

---

Query: Leucine-rich repeat-like protein (SEQ ID 4)  
Sbjct: sp|R85888|R85888 WD-40 domain-contg. insulin-like growth factor binding protein (SEQ ID NO: 22)  
Length = 605

Score = 293 (103.1 bits), Expect = 7.3e-22, P = 7.3e-22  
Identities = 141/467 (30%), Positives = 208/467 (44%)

Query: 198 GFHFLITVGWRNRSGTATAASQGYCKLVGGACRGOSLASV-PSSLPPhARM--LTIDAN 368  
Sbjct: G L + N S AA Q + L G + +G L S + P +L + L L+ N 75 GTQALWLDGNMNLSSVPPAFAFQNLSSL--GFUNLQGGQLGSLEPOAALGLENLICHLHERN 132

Query: 369 PLKTLWNHSIQLQPYPLIESISLHSCHLERISRGAFOEQHRLSLVLDNCLSENYEETAA 548  
Sbjct: L+L LR L+L+GN L AL L+ LR + L+ N + + +VF L RL++L 133 QLRSLAGTFAHTPALASIGLISNNRISRLEDGLFEGLGLSLWDLNLGWNSLAVLPD---AA 189

Query: 549 LHALPGRRLDLSIGNALTEDMAIMIQNLSSLRSVLAGNTIMRLDSVFEGLERIRELD 728  
Sbjct: 190 FRGLGSRELVLAGNRLAYLQPALF-SGLAELRELDISNALRAKANVVFVQLPRLQKLY 248

Query: 729 LQRNYTIEIEGGAFDGLAELRHNLAFNNLPCIVD-F-GLTRLRVLUVNSYNLWFLAT 899  
Sbjct: 249 LDRNLIAAVAPGAFLGKALRWDLISHNRAVAGILEDTPGGLLGLRVRLRSHNAIASLRRP 308

Query: 900 GGEAAFFELETDLSSHNLQ--LFPLLPOYSKIRTILLRDNNM-----GFYRDLYNTSSPR 1058  
Sbjct: 309 TFKDLHFILEELQQLGHNRIRQLAERSFEGFLGIGOLEVLTIDHNLQEVKAGFLGTINVA--- 365

Query: 1059 EMVAQFLILVDGNVTNTTSLWEFFSSDIADLRFIDMSQNOFOYIPDGFLRKMPSLSHL 1238  
Sbjct: 366 -----VNNSLSCNCLR---NLPEQVFRG-LGKIHSLHILRGSCIGRIRPHFTGIGSLRL 415

Query: 1239 NLHQNCUMLTILHIREHPPGALTDLDSHNLQISLCPLPAASDRVGGPPSCVDFRNMASIRSISLFCGCGILGAL 1418  
Sbjct: L N I+ + + L ELDL+ NQL+ HL L LG L LS N+L + 416 FLKDNGLGVGIEEQSLWGLAELLELDLTSNQLT--HLPHRLFQGLGKLEYLLISRNRLAEL 473

Query: 1419 PPGIFANARNITLDMSHNQIISLCPLPAASDRVGGPPSCVDFRNMASIRSISLFCGCGILGAL 1598  
Sbjct: P + LD+SHN++ P + +G + RN SLR+ + GL L 474 PADALGFLQRAFWLDVSHNRLALPNSLLAP-LGRLRYLISLRNN-SRTFTPOPPGLRL 531

**FIG. 4**

## DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

As [a] below named inventor(s), I/we hereby declare that:

**Bryan J. Boyle, George Yeung, Nancy K. Mize, Matthew C. Arterburn, Y. Tom Tang, Chenghua Liu, Radoje T. Drmanac, Menq-Yun Wang, Lichuan Chen, Yea-Huey Yang**

My/our residence, post office address and citizenship is/are as stated below next to my/our name(s).

I/we believe I/we am/are an/the original, first and sole/joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled: METHODS AND MATERIALS RELATING TO LEUCINE-RICH REPEAT PROTEIN-LIKE (LRR PROTEIN-LIKE) POLYPEPTIDES AND POLYNUCLEOTIDES, the specification of which

X is attached hereto.

— was filed on [date] as Application Serial Number [ ] and was amended on [date].

I/We hereby state that I/we have reviewed and understand the contents of the above-identified specification, including the claims as amended by any amendment referred to above.

I/We acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56(a).

I/We hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate, listed below and so identified, and I/we have also identified below any foreign application for patent or inventor's certificate on this invention filed by me or my legal representatives or assigns and having a filing date before that of the application on which priority is claimed:

NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	PRIORITY CLAIMED - YES OR NO

I/We hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I/we acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

SERIAL NUMBER	FILING DATE	STATUS
09/560,875	April 27, 2000	Pending
09/496,914	February 03, 2000	Pending

I/We hereby declare that all statements made herein of my/our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I/We hereby appoint the following attorneys and agents, with full power of substitution and revocation, to prosecute this application and to transact all business in the United States Patent and Trademark Office connected therewith and request that all correspondence and telephone calls with respect to this application be directed to Leslie A. Mooi, HYSEQ, INC., 670 Almanor Avenue, Sunnyvale, CA 94085, Telephone No. (408) 524-8100:

ATTORNEY	REGISTRATION NO.
Petrina S. Hsi	38,496
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Full name of  
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Inventor's signature: \_\_\_\_\_ Date: \_\_\_\_\_

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Citizenship: United States of America

Full name of  
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Inventor's signature: \_\_\_\_\_ Date: \_\_\_\_\_

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Citizenship: United States of America

Full name of  
sixth joint inventor: Chenghua Liu

Inventor's signature: \_\_\_\_\_ Date: \_\_\_\_\_

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Full name of  
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Inventor's signature: \_\_\_\_\_ Date: \_\_\_\_\_

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Citizenship: Yugoslavia

Full name of  
eighth joint inventor: Menq-Yun Wang

Inventor's signature: \_\_\_\_\_ Date: \_\_\_\_\_

Residence and Post  
Office Address: 19300 Chablis Court, Saratoga, CA 95070

Citizenship: United States of America

Full name of  
ninth joint inventor: Lichuan Chen

Inventor's signature: \_\_\_\_\_ Date: \_\_\_\_\_

Residence and Post  
Office Address: 1266 Lime Drive, Sunnyvale, CA 94087

Citizenship: United States of America

Full name of  
tenth joint inventor: Yea-Huey Yang

Inventor's signature: \_\_\_\_\_ Date: \_\_\_\_\_

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Citizenship: Taiwan

SEQUENCE LISTING

<110> Boyle, Bryan J  
Yeung, George  
Mize, Nancy K  
Arterburn, Matthew C  
Tang, Y. Tom  
Liu, Chenghua  
Drmanac, Radoje T  
Wang, Meng-Yun  
Chen, Lichuan  
Yang, Yea-Huey

<120> METHODS AND MATERIALS RELATING TO LEUCINE-RICH REPEAT PROTEIN-LIKE (LRR PROTEIN-LIKE) POLYPEPTIDES AND POLYNUCLEOTIDES

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gctgtgttgc ttccaggaaat atggcatctt taaggagctt gtctctggag ggctggccct	1260
gggggcatttccgactgccc attccaaggg acctccctga ctacttagac tctcaagcac	1320
tgggggtctt gaaagggagc ctgcggactt ccaagatgtt cccatgtac agggctgttt	1380
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 <213> Homo sapiens  
  
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 aaggaggtag cccagccccg cgttcggtcg ctctcgagga ggccggagcc cccggagacg 120  
 atgcgccccg cgagccgcct ggcctgcgg gagcggctgc ccttcat atg gag ttg 176  
 Met Glu Leu  
 1  
  
 ctg cct ctt tgg ctc tgc ctg ggt ttt cac ttc ctg acc gtg ggc tgg 224  
 Leu Pro Leu Trp Leu Cys Leu Gly Phe His Phe Leu Thr Val Gly Trp  
 5 10 15  
  
 agg aac aga agc gga aca gcc aca gca gcc tcc caa gga gtc tgc aag 272  
 Arg Asn Arg Ser Gly Thr Ala Thr Ala Ala Ser Gln Gly Val Cys Lys  
 20 25 30 35  
  
 ttg gtg ggt gga gcc gct gac tgc cga ggg cag agc ctc gct tcg gtg 320  
 Leu Val Gly Gly Ala Ala Asp Cys Arg Gly Gln Ser Leu Ala Ser Val  
 40 45 50  
  
 ccc agc agc ctc ccg ccc cac gcc cggt atg ctc acc ctg gat gcc aac 368  
 Pro Ser Ser Leu Pro Pro His Ala Arg Met Leu Thr Leu Asp Ala Asn  
 55 60 65  
  
 cct ctc aag acc ctg tgg aat cac tcc ctc cag cct tac cct ctc ctg 416  
 Pro Leu Lys Thr Leu Trp Asn His Ser Leu Gln Pro Tyr Pro Leu Leu  
 70 75 80  
  
 gag agc ctc agc ctg cac agc tgc cac ctg gag cgc atc agc cgc ggc 464  
 Glu Ser Leu Ser Leu His Ser Cys His Leu Glu Arg Ile Ser Arg Gly  
 85 90 95  
  
 gcc ttc cag gag caa ggt cac ctg cgc agc ctg gtc ctg ggg gac aac 512  
 Ala Phe Gln Glu Gln Gly His Leu Arg Ser Leu Val Leu Gly Asp Asn  
 100 105 110 115  
  
 tgc ctc tca gag aac tac gaa gag acg gca gcc gcc ctc cac gcc ctg 560  
 Cys Leu Ser Glu Asn Tyr Glu Glu Thr Ala Ala Leu His Ala Leu  
 120 125 130  
  
 ccg ggc ctg cgg agg ctg gac ttg tca gga aac gcc ctg acg gag gac 608  
 Pro Gly Leu Arg Arg Leu Asp Leu Ser Gly Asn Ala Leu Thr Glu Asp  
 135 140 145  
  
 atg gca gcg ctc atg ctc cag aac ctc tcc tcg ctg cgg tcc gtg tcc 656  
 Met Ala Ala Leu Met Leu Gln Asn Leu Ser Ser Leu Arg Ser Val Ser  
 150 155 160  
  
 ctg gcg ggg aac acc atc atg cgg ctg gac gac tcc gtc ttc gag ggc 704

Leu Ala Gly Asn Thr Ile Met Arg Leu Asp Asp Ser Val Phe Glu Gly			
165	170	175	
ctg gag cgt ctc cgg gag ctg gat ctg cag agg aac tac atc ttc gag		752	
Leu Glu Arg Leu Arg Glu Leu Asp Leu Gln Arg Asn Tyr Ile Phe Glu			
180	185	190	195
atc gag ggc ggc gct ttc gac ggc ctg gct gag ctg agg cac ctc aac		800	
Ile Glu Gly Gly Ala Phe Asp Gly Leu Ala Glu Leu Arg His Leu Asn			
200	205	210	
ctg gcc ttc aac aac ctc ccc tgc atc gtg gac ttc ggg ctc acg cgg		848	
Leu Ala Phe Asn Asn Leu Pro Cys Ile Val Asp Phe Gly Leu Thr Arg			
215	220	225	
ctg cgg gtc ctc aac gtc agc tac aac gtc ctg gag tgg ttc ctc gcg		896	
Leu Arg Val Leu Asn Val Ser Tyr Asn Val Leu Glu Trp Phe Leu Ala			
230	235	240	
acc ggg gga gag gct gcc ttc gag ctg gag acg ctg gac ctg tct cac		944	
Thr Gly Gly Ala Ala Phe Glu Leu Glu Thr Leu Asp Leu Ser His			
245	250	255	
aac cag ctg ctg ttc ttc ccg ctg ccc cag tac agc aag ttg cgg		992	
Asn Gln Leu Leu Phe Pro Leu Leu Pro Gln Tyr Ser Lys Leu Arg			
260	265	270	275
acc ctc ctg ctg cgc gac aac aac atg ggc ttc tac cgg gac ctg tac		1040	
Thr Leu Leu Leu Arg Asp Asn Asn Met Gly Phe Tyr Arg Asp Leu Tyr			
280	285	290	
aac acc tcg tcg ccg agg gag atg gtg gcc cag ttc ctc ctc gtg gac		1088	
Asn Thr Ser Ser Pro Arg Glu Met Val Ala Gln Phe Leu Leu Val Asp			
295	300	305	
ggc aac gtg acc aac atc acc acc gtc agc ctc tgg gaa gaa ttc tcc		1136	
Gly Asn Val Thr Asn Ile Thr Thr Val Ser Leu Trp Glu Glu Phe Ser			
310	315	320	
tcc agc gac ctc gca gat ctc cgc ttc ctg gac atg agc cag aac cag		1184	
Ser Ser Asp Leu Ala Asp Leu Arg Phe Leu Asp Met Ser Gln Asn Gln			
325	330	335	
tcc cag tac ctg cca gac ggc ttc ctg agg aaa atg cct tcc ctc tcc		1232	
Phe Gln Tyr Leu Pro Asp Gly Phe Leu Arg Lys Met Pro Ser Leu Ser			
340	345	350	355
cac ctg aac ctc cac cag aat tgc ctg atg acg ctt cac att cgg gag		1280	
His Leu Asn Leu His Gln Asn Cys Leu Met Thr Leu His Ile Arg Glu			
360	365	370	
cac gag ccc ccc gga gcg ctc acc gag ctg gac ctg agc cac aac cag		1328	
His Glu Pro Pro Gly Ala Leu Thr Glu Leu Asp Leu Ser His Asn Gln			
375	380	385	
ctg tcg gag ctg cac ctg gct ccg ggg ctg gcc agc tgc ctg ggc agc		1376	
Leu Ser Glu Leu His Leu Ala Pro Gly Leu Ala Ser Cys Leu Gly Ser			
390	395	400	
ctg cgc ttg ttc aac ctg agc tcc aac cag ctc ctg ggc gtc ccc cct		1424	

Leu Arg Leu Phe Asn Leu Ser Ser Asn Gln Leu Leu Gly Val Pro Pro			
405	410	415	
ggc ctc ttc gcc aat gct agg aac atc act aca ctt gac atg agc cac		1472	
Gly Leu Phe Ala Asn Ala Arg Asn Ile Thr Thr Leu Asp Met Ser His			
420	425	430	435
aat cag atc tca ctt tgt ccc ctg cca gct gcc tcg gac cggt ggc		1520	
Asn Gln Ile Ser Leu Cys Pro Leu Pro Ala Ala Ser Asp Arg Val Gly			
440	445	450	
ccc cct agc tgt gtg gat ttc agg aat atg gca tct tta agg agc ctg		1568	
Pro Pro Ser Cys Val Asp Phe Arg Asn Met Ala Ser Leu Arg Ser Leu			
455	460	465	
tct ctg gag ggc tgt ggc ctg ggg gca ttg cca gac tgc cca ttc caa		1616	
Ser Leu Glu Gly Cys Gly Leu Gly Ala Leu Pro Asp Cys Pro Phe Gln			
470	475	480	
ggg acc tcc ctg acc tac tta gac ctc tca agc aac tgg ggg gtt ctg		1664	
Gly Thr Ser Leu Thr Tyr Leu Asp Leu Ser Ser Asn Trp Gly Val Leu			
485	490	495	
aat ggg agc ctc gcc cca ctc cag gat gtt gcc ccc atg tta cag gtc		1712	
Asn Gly Ser Leu Ala Pro Leu Gln Asp Val Ala Pro Met Leu Gln Val			
500	505	510	515
ctg tct ctc agg aac atg ggc ctc cac tcc agc ttt atg gcg ttg gac		1760	
Leu Ser Leu Arg Asn Met Gly Leu His Ser Ser Phe Met Ala Leu Asp			
520	525	530	
ttc tct ggg ttt ggg aat ctc agg gac tta gat ctg tcg ggg aat tgc		1808	
Phe Ser Gly Phe Gly Asn Leu Arg Asp Leu Asp Leu Ser Gly Asn Cys			
535	540	545	
ttg ccc atc ttc cca agg ttt ggg ggc agc ctg gcc ctg gag acc ctg		1856	
Leu Pro Ile Phe Pro Arg Phe Gly Gly Ser Leu Ala Leu Glu Thr Leu			
550	555	560	
gat ctc cgt aga aac tcg ctc aca gcc ctt ccc cag aag gct gtg tct		1904	
Asp Leu Arg Arg Asn Ser Leu Thr Ala Leu Pro Gln Lys Ala Val Ser			
565	570	575	
gag cag ctc tcg aga ggt ctg cgg acc atc tac ctc agt cag aat cca		1952	
Glu Gln Leu Ser Arg Gly Leu Arg Thr Ile Tyr Leu Ser Gln Asn Pro			
580	585	590	595
tat gac tgc tgt ggg gtg gat ggc tgg ggg gcc ctg cag cat ggg cag		2000	
Tyr Asp Cys Cys Gly Val Asp Gly Trp Gly Ala Leu Gln His Gly Gln			
600	605	610	
acg gtg gcc gac tgg gcc atg gtc acc tgc aac ctc tcc tcc aag atc		2048	
Thr Val Ala Asp Trp Ala Met Val Thr Cys Asn Leu Ser Ser Lys Ile			
615	620	625	
atc cgc gtg acg gag ctg ccc gga ggt gtg cct cgg gac tgc aag tgg		2096	
Ile Arg Val Thr Glu Leu Pro Gly Gly Val Pro Arg Asp Cys Lys Trp			
630	635	640	
gag cgg ctg gac ctg ggc ctg ctc tac ctc gtg ctc atc ctc ccc agc		2144	

Glu Arg Leu Asp Leu Gly Leu Leu Tyr Leu Val Leu Ile Leu Pro Ser			
645	650	655	
tgc ctc acc ctg ctg gtg gcc tgc act gtc atc gtc ctc act ttt aag			2192
Cys Leu Thr Leu Leu Val Ala Cys Thr Val Ile Val Leu Thr Phe Lys			
660	665	670	675
aag cct ctg ctt cag gtc atc aag agc cgc tgc cac tgg tcc tcc gtt			2240
Lys Pro Leu Leu Gln Val Ile Lys Ser Arg Cys His Trp Ser Ser Val			
680	685	690	
tac tga cctggctgtg tgccaagact cgaaattcgg tccgcacaca acaggacact			2296
Tyr			

ttctctgccca gctttcaaga tgtgatgcag aggccaagtc tgacgaattt aagtttcaat			2356
taaaatctaa tatgtttcca taactcatct gcccacacca cgcatgtatt cggccgctct			2416
agaggatcct tgccctaagtc ga			2438

<210> 4  
<211> 692  
<212> PRT  
<213> Homo sapiens

<400> 4

Met Glu Leu Leu Pro Leu Trp Leu Cys Leu Gly Phe His Phe Leu Thr			
1	5	10	15

Val Gly Trp Arg Asn Arg Ser Gly Thr Ala Thr Ala Ala Ser Gln Gly			
20	25	30	

Val Cys Lys Leu Val Gly Gly Ala Ala Asp Cys Arg Gly Gln Ser Leu			
35	40	45	

Ala Ser Val Pro Ser Ser Leu Pro Pro His Ala Arg Met Leu Thr Leu			
50	55	60	

Asp Ala Asn Pro Leu Lys Thr Leu Trp Asn His Ser Leu Gln Pro Tyr			
65	70	75	80

Pro Leu Leu Glu Ser Leu Ser His Ser Cys His Leu Glu Arg Ile			
85	90	95	

Ser Arg Gly Ala Phe Gln Glu Gln Gly His Leu Arg Ser Leu Val Leu			
100	105	110	

Gly Asp Asn Cys Leu Ser Glu Asn Tyr Glu Glu Thr Ala Ala Leu			
115	120	125	

His Ala Leu Pro Gly Leu Arg Arg Leu Asp Leu Ser Gly Asn Ala Leu  
130 135 140

Thr Glu Asp Met Ala Ala Leu Met Leu Gln Asn Leu Ser Ser Leu Arg  
145 150 155 160

Ser Val Ser Leu Ala Gly Asn Thr Ile Met Arg Leu Asp Asp Ser Val  
165 170 175

Phe Glu Gly Leu Glu Arg Leu Arg Glu Leu Asp Leu Gln Arg Asn Tyr  
180 185 190

Ile Phe Glu Ile Glu Gly Gly Ala Phe Asp Gly Leu Ala Glu Leu Arg  
195 200 205

His Leu Asn Leu Ala Phe Asn Asn Leu Pro Cys Ile Val Asp Phe Gly  
210 215 220

Leu Thr Arg Leu Arg Val Leu Asn Val Ser Tyr Asn Val Leu Glu Trp  
225 230 235 240

Phe Leu Ala Thr Gly Gly Glu Ala Ala Phe Glu Leu Glu Thr Leu Asp  
245 250 255

Leu Ser His Asn Gln Leu Leu Phe Phe Pro Leu Leu Pro Gln Tyr Ser  
260 265 270

Lys Leu Arg Thr Leu Leu Arg Asp Asn Asn Met Gly Phe Tyr Arg  
275 280 285

Asp Leu Tyr Asn Thr Ser Ser Pro Arg Glu Met Val Ala Gln Phe Leu  
290 295 300

Leu Val Asp Gly Asn Val Thr Asn Ile Thr Thr Val Ser Leu Trp Glu  
305 310 315 320

Glu Phe Ser Ser Ser Asp Leu Ala Asp Leu Arg Phe Leu Asp Met Ser  
325 330 335

Gln Asn Gln Phe Gln Tyr Leu Pro Asp Gly Phe Leu Arg Lys Met Pro  
340 345 350

Ser Leu Ser His Leu Asn Leu His Gln Asn Cys Leu Met Thr Leu His  
355 360 365

Ile Arg Glu His Glu Pro Pro Gly Ala Leu Thr Glu Leu Asp Leu Ser  
370 375 380

His Asn Gln Leu Ser Glu Leu His Leu Ala Pro Gly Leu Ala Ser Cys  
385 390 395 400

Leu Gly Ser Leu Arg Leu Phe Asn Leu Ser Ser Asn Gln Leu Leu Gly  
405 410 415

Val Pro Pro Gly Leu Phe Ala Asn Ala Arg Asn Ile Thr Thr Leu Asp  
420 425 430

Met Ser His Asn Gln Ile Ser Leu Cys Pro Leu Pro Ala Ala Ser Asp  
435 440 445

Arg Val Gly Pro Pro Ser Cys Val Asp Phe Arg Asn Met Ala Ser Leu  
450 455 460

Arg Ser Leu Ser Leu Glu Gly Cys Gly Leu Gly Ala Leu Pro Asp Cys  
465 470 475 480

Pro Phe Gln Gly Thr Ser Leu Thr Tyr Leu Asp Leu Ser Ser Asn Trp  
485 490 495

Gly Val Leu Asn Gly Ser Leu Ala Pro Leu Gln Asp Val Ala Pro Met  
500 505 510

Leu Gln Val Leu Ser Leu Arg Asn Met Gly Leu His Ser Ser Phe Met  
515 520 525

Ala Leu Asp Phe Ser Gly Phe Gly Asn Leu Arg Asp Leu Asp Leu Ser  
530 535 540

Gly Asn Cys Leu Pro Ile Phe Pro Arg Phe Gly Gly Ser Leu Ala Leu  
545 550 555 560

Glu Thr Leu Asp Leu Arg Arg Asn Ser Leu Thr Ala Leu Pro Gln Lys  
565 570 575

Ala Val Ser Glu Gln Leu Ser Arg Gly Leu Arg Thr Ile Tyr Leu Ser  
580 585 590

Gln Asn Pro Tyr Asp Cys Cys Gly Val Asp Gly Trp Gly Ala Leu Gln  
595 600 605

His Gly Gln Thr Val Ala Asp Trp Ala Met Val Thr Cys Asn Leu Ser  
610 615 620

Ser Lys Ile Ile Arg Val Thr Glu Leu Pro Gly Gly Val Pro Arg Asp  
625 630 635 640

Cys Lys Trp Glu Arg Leu Asp Leu Gly Leu Leu Tyr Leu Val Leu Ile  
645 650 655

Leu Pro Ser Cys Leu Thr Leu Leu Val Ala Cys Thr Val Ile Val Leu  
660 665 670

Thr Phe Lys Lys Pro Leu Leu Gln Val Ile Lys Ser Arg Cys His Trp  
675 680 685

Ser Ser Val Tyr  
690

<210> 5  
<211> 2079  
<212> DNA  
<213> Homo sapiens

<400> 5  
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aacagaagcg gaacagccac agcagcctcc caaggagctc gcaagttgggt gggtgagcc 120  
gctgactgcc gagggcagag cctcgcttcg gtgcccagca gcctcccgcc ccacgccccgg 180  
atgctcaccc tggatgccaa ccctctcaag accctgttggaa atcactccct ccagccttac 240  
cctctccctgg agagcctcag cctgcacagc tgccacctgg agcgcattcag ccgcggcgcc 300  
ttccaggagc aaggtaacct ggcgagccctg gtcctgggggg acaactgcct ctcagagaac 360  
tacgaagaga cggcagccgc cctccacgcctc ctggccggcc tgccggaggct ggacttgtca 420  
ggaaacgccc tgacggagga catggcagcg ctcatgctcc agaacctctc ctcgctgcgg 480  
tccgtgtccc tggcggggaa caccatcatg cggctggacg actccgtctt cgagggcctg 540  
gagcgtctcc gggagcttggaa tctgcagagg aactacatct tcgagatcga gggcggcgct 600  
ttcgacggcc tggctgagct gaggcacctc aacctggctc tcaacaacct cccctgcattc 660  
gtggacttcg ggctcacgcg gctgcgggtc ctcaacgtca gctacaacgt cctggagtgg 720  
ttcctcgca cggggggaga ggctgccttc gagctggaga cgctggacct gtctcacaac 780  
cagctgtgt tcttccccct gctgccccag tacagcaagt tgccggaccct cctgctgcgc 840  
gacaacaaca tgggcttcta ccgggacctg tacaacacct cgtcgccgag ggagatggtg 900

gcccagttcc tcctcggtga cggcaacgtg accaacatca ccaccgtcag cctctggaa 960  
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 cagtacactgc cagacggctt cctgaggaaa atgccttccc tctcccacct gaacccac 1080  
 cagaattgcc ttagtgcgtc tcacattcgg gagcacgagc ccccccggagc gctcaccgag 1140  
 ctggacactga gccacaacca gctgtcgag ctgcacactgg ctccggggct ggccagctgc 1200  
 ctggcagcc tgcgttgcgtt caacactgagc tccaaccagc tcctgggcgt cccccctggc 1260  
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 tgtccctgc cagctgcctc ggaccgggtg ggccccccta gctgtgtgga tttcaggaat 1380  
 atggcatctt taaggaggct gtctctggag ggctgtggcc tgggggcatt gccagactgc 1440  
 ccattccaag ggacccctt gaccaactta gacctctcaa gcaactgggg ggttctgaat 1500  
 gggagcctcg ccccaactcca ggtatgttgc cccatgttac aggttctgtc tctcaggaac 1560  
 atgggcctcc actccagctt tatggcggtt gacttctctg ggtttggaa tctcagggac 1620  
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 cagctctcga gaggtctgctg gaccatctac ctcagtcaga atccatatga ctgctgtggg 1800  
 gtggatggct gggggccct gcagcatggg cagacgggtgg ccgactgggc catggtcacc 1860  
 tgcaacctct cctccaaagat catccgcgtg acggagctgc ccggaggtgt gcctcgggac 1920  
 tgcaagtggg agcggctgga cctggccctg ctctacactg tgctcatctt cccagctgc 1980  
 ctcaccctgc tggtggccctg cactgtcatac gtcctcaactt ttaagaagcc tctgcttcag 2040  
 gtcataaga gcccgtgcca ctggtcctcc gtttactga 2079

<210> 6  
 <211> 14  
 <212> PRT  
 <213> Homo sapiens

<400> 6

Leu Thr Glu Leu Asp Leu Ser His Asn Gln Leu Ser Glu Leu  
 1 5 10

<210> 7  
 <211> 14  
 <212> PRT  
 <213> Homo sapiens

<400> 7

Leu Arg Arg Leu Asp Leu Ser Gly Asn Ala Leu Thr Glu Asp

1 5 10

<210> 8  
<211> 14  
<212> PRT  
<213> Homo sapiens

<400> 8

Phe Gly Asn Leu Arg Asp Leu Asp Leu Ser Gly Asn Cys Leu  
1 5 10

<210> 9  
<211> 14  
<212> PRT  
<213> Homo sapiens

<400> 9

Pro Gly Ala Leu Thr Glu Leu Asp Leu Ser His Asn Gln Leu  
1 5 10

<210> 10  
<211> 14  
<212> PRT  
<213> Homo sapiens

<400> 10

Leu Glu Thr Leu Asp Leu Ser His Asn Gln Leu Leu Phe Phe  
1 5 10

<210> 11  
<211> 14  
<212> PRT  
<213> Homo sapiens

<400> 11

Leu Thr Arg Leu Arg Val Leu Asn Val Ser Tyr Asn Val Leu  
1 5 10

<210> 12  
<211> 14  
<212> PRT  
<213> Homo sapiens

<400> 12

Leu Glu Thr Leu Asp Leu Arg Arg Asn Ser Leu Thr Ala Leu  
1 5 10

<210> 13  
<211> 14  
<212> PRT  
<213> Homo sapiens

<400> 13

Met Pro Ser Leu Ser His Leu Asn Leu His Gln Asn Cys Leu

1 5 10

<210> 14  
<211> 14  
<212> PRT  
<213> Homo sapiens

<400> 14

Leu Glu Arg Leu Arg Glu Leu Asp Leu Gln Arg Asn Tyr Ile  
1 5 10

<210> 15  
<211> 18  
<212> PRT  
<213> Homo sapiens

<400> 15

Met Glu Leu Leu Pro Leu Trp Leu Cys Leu Gly Phe His Phe Leu Thr  
1 5 10 15

Val Gly

<210> 16  
<211> 26  
<212> PRT  
<213> Homo sapiens

<400> 16

Leu Gly Leu Leu Tyr Leu Val Leu Ile Leu Pro Ser Cys Leu Thr Leu  
1 5 10 15

Leu Val Ala Cys Thr Val Ile Val Leu Thr  
20 25

<210> 17  
<211> 674  
<212> PRT  
<213> Homo sapiens

<400> 17

Trp Arg Asn Arg Ser Gly Thr Ala Thr Ala Ala Ser Gln Gly Val Cys  
1 5 10 15

Lys Leu Val Gly Gly Ala Ala Asp Cys Arg Gly Gln Ser Leu Ala Ser  
20 25 30

Val Pro Ser Ser Leu Pro Pro His Ala Arg Met Leu Thr Leu Asp Ala  
35 40 45

Asn Pro Leu Lys Thr Leu Trp Asn His Ser Leu Gln Pro Tyr Pro Leu  
50 55 60

Leu Glu Ser Leu Ser Leu His Ser Cys His Leu Glu Arg Ile Ser Arg  
65 70 75 80

Gly Ala Phe Gln Glu Gln Gly His Leu Arg Ser Leu Val Leu Gly Asp  
85 90 95

Asn Cys Leu Ser Glu Asn Tyr Glu Glu Thr Ala Ala Ala Leu His Ala  
100 105 110

Leu Pro Gly Leu Arg Arg Leu Asp Leu Ser Gly Asn Ala Leu Thr Glu  
115 120 125

Asp Met Ala Ala Leu Met Leu Gln Asn Leu Ser Ser Leu Arg Ser Val  
130 135 140

Ser Leu Ala Gly Asn Thr Ile Met Arg Leu Asp Asp Ser Val Phe Glu  
145 150 155 160

Gly Leu Glu Arg Leu Arg Glu Leu Asp Leu Gln Arg Asn Tyr Ile Phe  
165 170 175

Glu Ile Glu Gly Gly Ala Phe Asp Gly Leu Ala Glu Leu Arg His Leu  
180 185 190

Asn Leu Ala Phe Asn Asn Leu Pro Cys Ile Val Asp Phe Gly Leu Thr  
195 200 205

Arg Leu Arg Val Leu Asn Val Ser Tyr Asn Val Leu Glu Trp Phe Leu  
210 215 220

Ala Thr Gly Gly Glu Ala Ala Phe Glu Leu Glu Thr Leu Asp Leu Ser  
225 230 235 240

His Asn Gln Leu Leu Phe Phe Pro Leu Leu Pro Gln Tyr Ser Lys Leu  
245 250 255

Arg Thr Leu Leu Leu Arg Asp Asn Asn Met Gly Phe Tyr Arg Asp Leu  
260 265 270

Tyr Asn Thr Ser Ser Pro Arg Glu Met Val Ala Gln Phe Leu Leu Val  
275 280 285

Asp Gly Asn Val Thr Asn Ile Thr Thr Val Ser Leu Trp Glu Glu Phe  
290 295 300

Ser Ser Ser Asp Leu Ala Asp Leu Arg Phe Leu Asp Met Ser Gln Asn  
305 310 315 320

Gln Phe Gln Tyr Leu Pro Asp Gly Phe Leu Arg Lys Met Pro Ser Leu  
325 330 335

Ser His Leu Asn Leu His Gln Asn Cys Leu Met Thr Leu His Ile Arg  
340 345 350

Glu His Glu Pro Pro Gly Ala Leu Thr Glu Leu Asp Leu Ser His Asn  
355 360 365

Gln Leu Ser Glu Leu His Leu Ala Pro Gly Leu Ala Ser Cys Leu Gly  
370 375 380

Ser Leu Arg Leu Phe Asn Leu Ser Ser Asn Gln Leu Leu Gly Val Pro  
385 390 395 400

Pro Gly Leu Phe Ala Asn Ala Arg Asn Ile Thr Thr Leu Asp Met Ser  
405 410 415

His Asn Gln Ile Ser Leu Cys Pro Leu Pro Ala Ala Ser Asp Arg Val  
420 425 430

Gly Pro Pro Ser Cys Val Asp Phe Arg Asn Met Ala Ser Leu Arg Ser  
435 440 445

Leu Ser Leu Glu Gly Cys Gly Leu Gly Ala Leu Pro Asp Cys Pro Phe  
450 455 460

Gln Gly Thr Ser Leu Thr Tyr Leu Asp Leu Ser Ser Asn Trp Gly Val  
465 470 475 480

Leu Asn Gly Ser Leu Ala Pro Leu Gln Asp Val Ala Pro Met Leu Gln  
485 490 495

Val Leu Ser Leu Arg Asn Met Gly Leu His Ser Ser Phe Met Ala Leu  
500 505 510

Asp Phe Ser Gly Phe Gly Asn Leu Arg Asp Leu Asp Leu Ser Gly Asn  
515 520 525

Cys Leu Pro Ile Phe Pro Arg Phe Gly Gly Ser Leu Ala Leu Glu Thr  
530 535 540

Leu Asp Leu Arg Arg Asn Ser Leu Thr Ala Leu Pro Gln Lys Ala Val  
545 550 555 560

Ser Glu Gln Leu Ser Arg Gly Leu Arg Thr Ile Tyr Leu Ser Gln Asn  
565 570 575

Pro Tyr Asp Cys Cys Gly Val Asp Gly Trp Gly Ala Leu Gln His Gly  
580 585 590

Gln Thr Val Ala Asp Trp Ala Met Val Thr Cys Asn Leu Ser Ser Lys  
595 600 605

Ile Ile Arg Val Thr Glu Leu Pro Gly Gly Val Pro Arg Asp Cys Lys  
610 615 620

Trp Glu Arg Leu Asp Leu Gly Leu Leu Tyr Leu Val Leu Ile Leu Pro  
625 630 635 640

Ser Cys Leu Thr Leu Leu Val Ala Cys Thr Val Ile Val Leu Thr Phe  
645 650 655

Lys Lys Pro Leu Leu Gln Val Ile Lys Ser Arg Cys His Trp Ser Ser  
660 665 670

Val Tyr

<210> 18  
<211> 121  
<212> PRT  
<213> Homo sapiens  
  
<400> 18

Val Ser Leu Ala Gly Asn Thr Ile Met Arg Leu Asp Asp Ser Val Phe  
1 5 10 15

Glu Gly Leu Glu Arg Leu Arg Glu Leu Asp Leu Gln Arg Asn Tyr Ile  
20 25 30

Phe Glu Ile Glu Gly Ala Phe Asp Gly Leu Ala Glu Leu Arg His  
35 40 45

Leu Asn Leu Ala Phe Asn Asn Leu Pro Cys Ile Val Asp Phe Gly Leu  
50 55 60

Thr Arg Leu Arg Val Leu Asn Val Ser Tyr Asn Val Leu Glu Trp Phe  
65 70 75 80

Leu Ala Thr Gly Gly Glu Ala Ala Phe Glu Leu Glu Thr Leu Asp Leu  
85 90 95

Ser His Asn Gln Leu Leu Phe Phe Pro Leu Leu Pro Gln Tyr Ser Lys  
100 105 110

Leu Arg Thr Leu Leu Leu Arg Asp Asn  
115 120

<210> 19  
<211> 626  
<212> PRT  
<213> Homo sapiens

Cys Lys Met Val Asp Lys Lys Val Ser Cys Gln Val Leu Gly Leu Leu  
1 5 10 15

Gln Val Pro Ser Val Leu Pro Pro Asp Thr Glu Thr Leu Asp Leu Ser  
20 25 30

Gly Asn Gln Leu Arg Ser Ile Leu Ala Ser Pro Leu Gly Phe Tyr Thr  
35 40 45

Ala Leu Arg His Leu Asp Leu Ser Thr Asn Glu Ile Ser Phe Leu Gln  
50 55 60

Pro Gly Ala Phe Gln Ala Leu Thr His Leu Glu His Leu Ser Leu Ala  
65 70 75 80

His Asn Arg Leu Ala Met Ala Thr Ala Leu Ser Ala Gly Gly Leu Gly  
85 90 95

Pro Leu Pro Arg Val Thr Ser Leu Asp Leu Ser Gly Asn Ser Leu Tyr  
100 105 110

Ser Gly Leu Leu Glu Arg Leu Leu Gly Glu Ala Pro Ser Leu His Thr  
115 120 125

Leu Ser Leu Ala Glu Asn Ser Leu Thr Arg Leu Thr Arg His Thr Phe  
130 135 140

Arg Asp Met Pro Ala Leu Glu Gln Leu Asp Leu His Ser Asn Val Leu

145                    150                    155                    160  
Met Asp Ile Glu Asp Gly Ala Phe Glu Gly Leu Pro Arg Leu Thr His  
165                    170                    175  
Leu Asn Leu Ser Arg Asn Ser Leu Thr Cys Ile Ser Asp Phe Ser Leu  
180                    185                    190  
Gln Gln Leu Arg Val Leu Asp Leu Ser Cys Asn Ser Ile Glu Ala Phe  
195                    200                    205  
Gln Thr Ala Ser Gln Pro Gln Ala Glu Phe Gln Leu Thr Trp Leu Asp  
210                    215                    220  
Leu Arg Glu Asn Lys Leu Leu His Phe Pro Asp Leu Ala Ala Leu Pro  
225                    230                    235                    240  
Arg Leu Ile Tyr Leu Asn Leu Ser Asn Asn Leu Ile Arg Leu Pro Thr  
245                    250                    255  
Gly Pro Pro Gln Asp Ser Lys Gly Ile His Ala Pro Ser Glu Gly Trp  
260                    265                    270  
Ser Ala Leu Pro Leu Ser Ala Pro Ser Gly Asn Ala Ser Gly Arg Pro  
275                    280                    285  
Leu Ser Gln Leu Leu Asn Leu Asp Leu Ser Tyr Asn Glu Ile Glu Leu  
290                    295                    300  
Ile Pro Asp Ser Phe Leu Glu His Leu Thr Ser Leu Cys Phe Leu Asn  
305                    310                    315                    320  
Leu Ser Arg Asn Cys Leu Arg Thr Phe Glu Ala Arg Arg Leu Gly Ser  
325                    330                    335  
Leu Pro Cys Leu Met Leu Leu Asp Leu Ser His Asn Ala Leu Glu Thr  
340                    345                    350  
Leu Glu Leu Gly Ala Arg Ala Leu Gly Ser Leu Arg Thr Leu Leu Leu  
355                    360                    365  
Gln Gly Asn Ala Leu Arg Asp Leu Pro Pro Tyr Thr Phe Ala Asn Leu  
370                    375                    380  
Ala Ser Leu Gln Arg Leu Asn Leu Gln Gly Asn Arg Val Ser Pro Cys  
385                    390                    395                    400  
Gly Gly Pro Asp Glu Pro Gly Pro Ser Gly Cys Val Ala Phe Ser Gly  
405                    410                    415  
Ile Thr Ser Leu Arg Ser Leu Ser Leu Val Asp Asn Glu Ile Glu Leu  
420                    425                    430  
Leu Arg Ala Gly Ala Phe Leu His Thr Pro Leu Thr Glu Leu Asp Leu  
435                    440                    445  
Ser Ser Asn Pro Gly Leu Glu Val Ala Thr Gly Ala Leu Gly Gly Leu  
450                    455                    460  
Glu Ala Ser Leu Glu Val Leu Ala Leu Gln Gly Asn Gly Leu Met Val

465	470	475	480
Leu Gln Val Asp Leu Pro Cys Phe Ile Cys Leu Lys Arg Leu Asn Leu			
485	490	495	
Ala Glu Asn Arg Leu Ser His Leu Pro Ala Trp Thr Gln Ala Val Ser			
500	505	510	
Leu Glu Val Leu Asp Leu Arg Asn Asn Ser Phe Ser Leu Leu Pro Gly			
515	520	525	
Ser Ala Met Gly Gly Leu Glu Thr Ser Leu Arg Arg Leu Tyr Leu Gln			
530	535	540	
Gly Asn Pro Leu Ser Cys Cys Gly Asn Gly Trp Leu Ala Ala Gln Leu			
545	550	555	560
His Gln Gly Arg Val Asp Val Asp Ala Thr Gln Asp Leu Ile Cys Arg			
565	570	575	
Phe Ser Ser Gln Glu Glu Val Ser Leu Ser His Val Arg Pro Glu Asp			
580	585	590	
Cys Glu Lys Gly Gly Leu Lys Asn Ile Asn Leu Ile Ile Ile Leu Thr			
595	600	605	
Phe Ile Leu Val Ser Ala Ile Leu Leu Thr Thr Leu Ala Ala Cys Cys			
610	615	620	
Cys Val			
625			
<210> 20			
<211> 510			
<212> PRT			
<213> Baboon			
<400> 20			
Pro Arg Ser Leu Glu Gly Ala Glu Pro Gly Thr Pro Gly Glu Ala Glu			
1	5	10	15
Gly Pro Ala Cys Pro Ala Thr Cys Ala Cys Ser Tyr Asp Asp Glu Val			
20	25	30	
Asn Glu Leu Ser Val Phe Cys Ser Ser Arg Asn Leu Thr Arg Leu Pro			
35	40	45	
Asp Gly Ile Pro Gly Gly Thr Gln Ala Leu Trp Leu Asp Ser Asn Asn			
50	55	60	
Leu Ser Ser Ile Pro Pro Ala Ala Phe Arg Asn Leu Ser Ser Leu Ala			
65	70	75	80
Phe Leu Asn Leu Gln Gly Gly Gln Leu Gly Ser Leu Glu Pro Gln Ala			
85	90	95	
Leu Leu Gly Leu Glu Asn Leu Cys His Leu His Leu Glu Arg Asn Gln			
100	105	110	

Leu Arg Ser Leu Ala Val Gly Thr Phe Ala Tyr Thr Pro Ala Leu Ala  
115 120 125

Leu Leu Gly Leu Ser Asn Asn Arg Leu Ser Arg Leu Glu Asp Gly Leu  
130 135 140

Phe Glu Gly Leu Gly Asn Leu Trp Asp Leu Asn Leu Gly Trp Asn Ser  
145 150 155 160

Leu Ala Val Leu Pro Asp Ala Ala Phe Arg Gly Leu Gly Gly Leu Arg  
165 170 175

Glu Leu Val Leu Ala Gly Asn Arg Leu Ala Tyr Leu Gln Pro Ala Leu  
180 185 190

Phe Ser Gly Leu Ala Glu Leu Arg Glu Leu Asp Leu Ser Arg Asn Ala  
195 200 205

Leu Arg Ala Ile Lys Ala Asn Val Phe Ala Gln Leu Pro Arg Leu Gln  
210 215 220

Lys Leu Tyr Leu Asp Arg Asn Leu Ile Ala Ala Val Ala Pro Gly Ala  
225 230 235 240

Phe Leu Gly Leu Lys Ala Leu Arg Trp Leu Asp Leu Ser His Asn Arg  
245 250 255

Val Ala Gly Leu Leu Glu Asp Thr Phe Pro Gly Leu Leu Gly Leu Arg  
260 265 270

Val Leu Arg Leu Ser His Asn Ala Ile Ala Ser Leu Arg Pro Arg Thr  
275 280 285

Phe Glu Asp Leu His Phe Leu Glu Glu Leu Gln Leu Gly His Asn Arg  
290 295 300

Ile Arg Gln Leu Ala Glu Arg Ser Phe Glu Gly Leu Gly Gln Leu Glu  
305 310 315 320

Val Leu Thr Leu Asp His Asn Gln Leu Gln Glu Val Lys Val Gly Ala  
325 330 335

Phe Leu Gly Leu Thr Asn Val Ala Val Met Asn Leu Ser Gly Asn Cys  
340 345 350

Leu Arg Asn Leu Pro Glu Gln Val Phe Arg Gly Leu Gly Lys Leu His  
355 360 365

Ser Leu His Leu Glu Gly Ser Cys Leu Gly Arg Ile Arg Pro His Thr  
370 375 380

Phe Ala Gly Leu Ser Gly Leu Arg Arg Leu Phe Leu Lys Asp Asn Gly  
385 390 395 400

Leu Val Gly Ile Glu Glu Gln Ser Leu Trp Gly Leu Ala Glu Leu Leu  
405 410 415

Glu Leu Asp Leu Thr Ser Asn Gln Leu Thr His Leu Pro His Gln Leu  
420 425 430

Phe Gln Gly Leu Gly Lys Leu Glu Tyr Leu Leu Leu Ser His Asn Arg  
435 440 445

Leu Ala Glu Leu Pro Ala Asp Ala Leu Gly Pro Leu Gln Arg Ala Phe  
450 455 460

Trp Leu Asp Val Ser His Asn Arg Leu Glu Ala Leu Pro Gly Ser Leu  
465 470 475 480

Leu Ala Ser Leu Gly Arg Leu Arg Tyr Leu Asn Leu Arg Asn Asn Ser  
485 490 495

Leu Arg Thr Phe Thr Pro Gln Pro Pro Gly Leu Glu Arg Leu  
500 505 510

<210> 21  
<211> 368  
<212> PRT  
<213> Homo sapiens

<400> 21

Gly Met Thr Val Leu Gln Arg Leu Met Ile Ser Asp Ser His Ile Ser  
1 5 10 15

Ala Val Ala Pro Gly Thr Phe Ser Asp Leu Ile Lys Leu Lys Thr Leu  
20 25 30

Arg Leu Ser Arg Asn Lys Ile Thr His Leu Pro Gly Ala Leu Leu Asp  
35 40 45

Lys Met Val Leu Leu Glu Gln Leu Phe Leu Asp His Asn Ala Leu Arg  
50 55 60

Gly Ile Asp Gln Asn Met Phe Gln Lys Leu Val Asn Leu Gln Glu Leu  
65 70 75 80

Ala Leu Asn Gln Asn Gln Leu Asp Phe Leu Pro Ala Ser Leu Phe Thr  
85 90 95

Asn Leu Glu Asn Leu Lys Leu Leu Asp Leu Ser Gly Asn Asn Leu Thr  
100 105 110

His Leu Pro Lys Gly Leu Leu Gly Ala Gln Ala Lys Leu Glu Arg Leu  
115 120 125

Leu Leu His Ser Asn Arg Leu Val Ser Leu Asp Ser Gly Leu Leu Asn  
130 135 140

Ser Leu Gly Ala Leu Thr Glu Leu Gln Phe His Arg Asn His Ile Arg  
145 150 155 160

Ser Ile Ala Pro Gly Ala Phe Asp Arg Leu Pro Asn Leu Ser Ser Leu  
165 170 175

Thr Leu Ser Arg Asn His Leu Ala Phe Leu Pro Ser Ala Leu Phe Leu  
180 185 190

His Ser His Asn Leu Thr Leu Leu Thr Leu Phe Glu Asn Pro Leu Ala  
195 200 205

Glu Leu Pro Gly Val Leu Phe Gly Glu Met Gly Gly Leu Gln Glu Leu  
210 215 220

Trp Leu Asn Arg Thr Gln Leu Arg Thr Leu Pro Ala Ala Ala Phe Arg  
225 230 235 240

Asn Leu Ser Arg Leu Arg Tyr Leu Gly Val Thr Leu Ser Pro Arg Leu  
245 250 255

Ser Ala Leu Pro Gln Gly Ala Phe Gln Gly Leu Gly Glu Leu Gln Val  
260 265 270

Leu Ala Leu His Ser Asn Gly Leu Thr Ala Leu Pro Asp Gly Leu Leu  
275 280 285

Arg Gly Leu Gly Lys Leu Arg Gln Val Ser Leu Arg Arg Asn Arg Leu  
290 295 300

Arg Ala Leu Pro Arg Ala Leu Phe Arg Asn Leu Ser Ser Leu Glu Ser  
305 310 315 320

Val Gln Leu Asp His Asn Gln Leu Glu Thr Leu Pro Gly Asp Val Phe  
325 330 335

Gly Ala Leu Pro Arg Leu Thr Glu Val Leu Leu Gly His Asn Ser Trp  
340 345 350

Arg Cys Asp Cys Gly Leu Gly Pro Phe Leu Gly Trp Leu Arg Gln His  
355 360 365

<210> 22

<211> 457

<212> PRT

<213> synthetic construct

<400> 22

Gly Thr Gln Ala Leu Trp Leu Asp Gly Asn Asn Leu Ser Ser Val Pro  
1 5 10 15

Pro Ala Ala Phe Gln Asn Leu Ser Ser Leu Gly Phe Leu Asn Leu Gln  
20 25 30

Gly Gly Gln Leu Gly Ser Leu Glu Pro Gln Ala Leu Leu Gly Leu Glu  
35 40 45

Asn Leu Cys His Leu His Leu Glu Arg Asn Gln Leu Arg Ser Leu Ala  
50 55 60

Leu Gly Thr Phe Ala His Thr Pro Ala Leu Ala Ser Leu Gly Leu Ser  
65 70 75 80

Asn Asn Arg Leu Ser Arg Leu Glu Asp Gly Leu Phe Glu Gly Leu Gly  
85 90 95

Ser Leu Trp Asp Leu Asn Leu Gly Trp Asn Ser Leu Ala Val Leu Pro  
100 105 110

Asp Ala Ala Phe Arg Gly Leu Gly Ser Leu Arg Glu Leu Val Leu Ala

115                    120                    125  
Gly Asn Arg Leu Ala Tyr Leu Gln Pro Ala Leu Phe Ser Gly Leu Ala  
130                    135                    140  
Glu Leu Arg Glu Leu Asp Leu Ser Arg Asn Ala Leu Arg Ala Ile Lys  
145                    150                    155                    160  
Ala Asn Val Phe Val Gln Leu Pro Arg Leu Gln Lys Leu Tyr Leu Asp  
165                    170                    175  
Arg Asn Leu Ile Ala Ala Val Ala Pro Gly Ala Phe Leu Gly Leu Lys  
180                    185                    190  
Ala Leu Arg Trp Leu Asp Leu Ser His Asn Arg Val Ala Gly Leu Leu  
195                    200                    205  
Glu Asp Thr Phe Pro Gly Leu Leu Gly Leu Arg Val Leu Arg Leu Ser  
210                    215                    220  
His Asn Ala Ile Ala Ser Leu Arg Pro Arg Thr Phe Lys Asp Leu His  
225                    230                    235                    240  
Phe Leu Glu Glu Leu Gln Leu Gly His Asn Arg Ile Arg Gln Leu Ala  
245                    250                    255  
Glu Arg Ser Phe Glu Gly Leu Gly Gln Leu Glu Val Leu Thr Leu Asp  
260                    265                    270  
His Asn Gln Leu Gln Glu Val Lys Ala Gly Ala Phe Leu Gly Leu Thr  
275                    280                    285  
Asn Val Ala Val Met Asn Leu Ser Gly Asn Cys Leu Arg Asn Leu Pro  
290                    295                    300  
Glu Gln Val Phe Arg Gly Leu Gly Lys Leu His Ser Leu His Leu Glu  
305                    310                    315                    320  
Gly Ser Cys Leu Gly Arg Ile Arg Pro His Thr Phe Thr Gly Leu Ser  
325                    330                    335  
Gly Leu Arg Arg Leu Phe Leu Lys Asp Asn Gly Leu Val Gly Ile Glu  
340                    345                    350  
Glu Gln Ser Leu Trp Gly Leu Ala Glu Leu Leu Glu Leu Asp Leu Thr  
355                    360                    365  
Ser Asn Gln Leu Thr His Leu Pro His Arg Leu Phe Gln Gly Leu Gly  
370                    375                    380  
Lys Leu Glu Tyr Leu Leu Ser Arg Asn Arg Leu Ala Glu Leu Pro  
385                    390                    395                    400  
Ala Asp Ala Leu Gly Pro Leu Gln Arg Ala Phe Trp Leu Asp Val Ser  
405                    410                    415  
His Asn Arg Leu Glu Ala Leu Pro Asn Ser Leu Leu Ala Pro Leu Gly  
420                    425                    430  
Arg Leu Arg Tyr Leu Ser Leu Arg Asn Asn Ser Leu Arg Thr Phe Thr

435

440

445

Pro Gln Pro Pro Gly Leu Glu Arg Leu  
450 455